TOSOH BIOSCIENCE

2014–2016 Process Product Guide

TOSOH

TOYOPEARL & TSKgel Bulk Resin

Experts in Chromatography

TOYOPEARL® Bulk Resin
TSKgel® Bulk Resin
EcoSEC® GPC System
TSKgel HPLC Columns

2014–2016 Product Guide

TOSOH BIOSCIENCE

Announcing the 9th HIC/RPC Bioseparation Conference in 2015!

ADVANCEMENTS, APPLICATIONS AND THEORY IN DOWNSTREAM PROCESSING
NEW IDEAS CREATE IMPROVED SOLUTIONS FOR DOWNSTREAM PROCESSING

The 9th HIC/RPC Bioseparation Conference will be held in Sliema, Malta from March 16-19, 2015. The conference is focused on the better understanding of the hydrophobic nature of biological targets and their chromatographic isolation and purification.

Visit www.hic-rpc.org for additional information.

Where to Order

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Contents


TOYOPEARL
HW-55F
Net Vol. 500mL
Part No. 07457
Lot No.
FOR IN VITRO USE ONLY
TOSOH
TOYOPEARL
HW-65F
Net Vol. 500mL
Part No. 07465
Lot No.
FOR IN VITRO USE ONLY
TOSOH
TOYOPEARL
HW-75F
Net Vol. 500mL
Part No. 07469
Lot No.
FOR IN VITRO USE ONLY
TOSOH

TOYOPEARL HW-40

TOYOPEARL HW-50

TOYOPEARL HW-55

TOYOPEARL HW-65

TOYOPEARL HW-75

The Role of Size Exclusion Chromatography in Process Purification

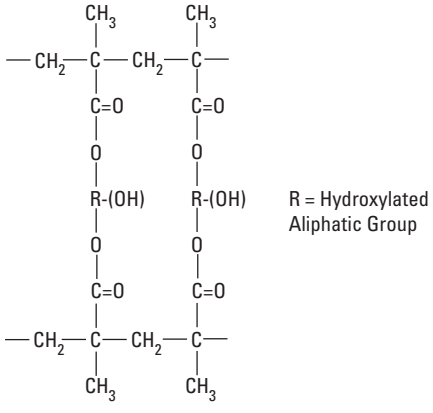
Size exclusion chromatography (SEC), also known as gel filtration chromatography, separates molecules in an aqueous mobile phase according to their hydrodynamic radius in solution as they pass through a porous structure. Molecules with a diameter greater than the largest pores within the resin are unable to enter the particle. Because they are excluded from the pores they travel quickly through the column and elute first. Smaller molecules, which are able to access pores within the resin particles, permeate a larger accessible volume within the column and are eluted later, in order of decreasing molar mass.

Because SEC has no adsorption capacity and its separation mechanism dilutes the sample during elution, it is not normally used in the capture or intermediate steps of manufacturing processes. It is most often used as a final polishing step where a target protein is being separated from its aggregates or other significantly different molar mass impurities. Another related application would be the desalting of the purified target protein in lieu of a more traditional diafiltration step.

TOYOPEARL HW SEC Resins

Tosoh Bioscience offers a number of TOYOPEARL HW resins for size exclusion chromatography (H = *hydrophilic,* W = *water-compatible)*. TOYOPEARL HW size exclusion resins are hydroxylated polymethacrylic polymer beads (Figure 1). Surface hydroxyl groups render these resins very hydrophilic, therefore minimal non-specific adsorption occurs, making the TOYOPEARL HW resins useful for protein separations. The semi-rigid polymeric nature and the narrow particle size distribution of these resins give them better pressure-flow properties than softer materials such as agarose. In addition, good mechanical stability of the TOYOPEARL HW resins produces excellent flow characteristics in large industrial size columns (up to 0.3 MPa).

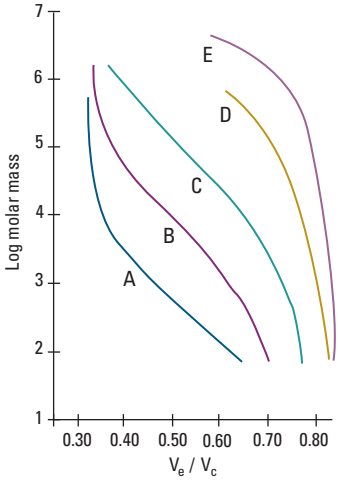
Figure 1: Resin Chemistry of TOYOPEARL HW resins (Hydroxylated Acrylic)



TOYOPEARL HW resins are chemically stable from pH 2-14. This allows a constant packing volume over a wide range of salt concentrations and cleaning in place (CIP) with acid or base. Also, these resins can be run at elevated temperatures (4 - 60 °C) and are autoclavable at 121 °C.

Commercial TOYOPEARL HW size exclusion resins are available in five pore sizes covering five different fractionation ranges, though there is some overlap among the listed ranges. The choice of TOYOPEARL HW resin depends on the molar mass of the feedstock components. Tables 1 and 2 show this information for proteins, dextrans and PEG (polyethylene glycol) polymers. The TOYOPEARL HW resin molar mass ranges span peptide and protein sizes between 100 to 5 x 10^7 daltons. Each TOYOPEARL HW resin exhibits a typical calibration curve and exclusion limit for globular proteins (Figure 2).

Figure 2: Calibration curves for globular proteins on TOYOPEARL resins

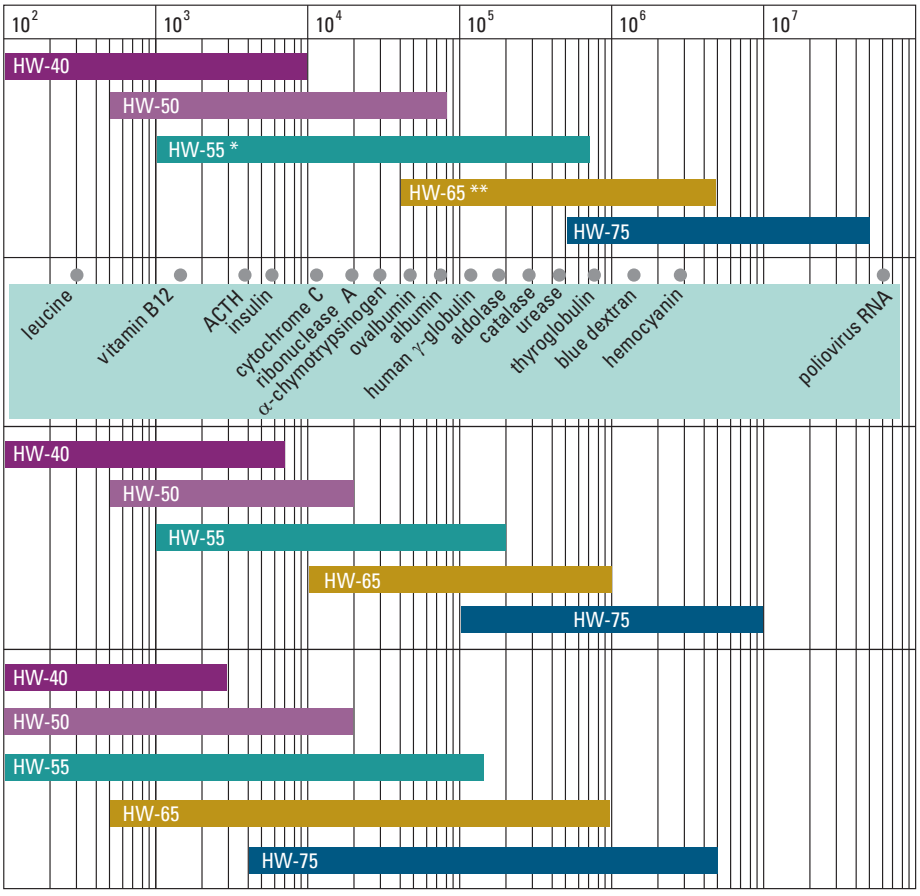


Resins:	**A. TOYOPEARL HW-40** **B. TOYOPEARL HW-50** **C. TOYOPEARL HW-55** **D. TOYOPEARL HW-65** **E. TOYOPEARL HW-75**
Column size:	22 mm ID × 30 cm
Mobile phase:	0.06 mol/L phosphate buffer, pH 7.0, + 0.06 mol/L KCl
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	protein standards
Legend:	V_e = elution volume V_c = column volume

Table 1: Properties and molar mass separation ranges of TOYOPEARL HW resins

			Molar mass of sample (Da)		
TOYOPEARL resin	**Particle size (µm)**	**Pore size (nm)**	**Polyethylene glycols and oxides**	**Dextrans**	**Globular proteins**
HW-40S HW-40F HW-40C	20 - 40 30 - 60 50 - 100	5	100 - 3,000	100 - 7,000	100 - 1×10^4
HW-50S HW-50F	20 - 40 30 - 60	12.5	100 - 1.8×10^4	500 - 2×10^4	500 - 8×10^4
HW-55S HW-55F	20 - 40 30 - 60	50	100 - 1.5×10^5	1,000 - 2×10^5	1,000 - 7×10^5
HW-65S HW-65F	20 - 40 30 - 60	100	500 - 1×10^6	1×10^4 - 1×10^6	4×10^4 - 5×10^6
HW-75F HW-75S	30 - 60	>100	4,000 - 5×10^6	1×10^5 - 1×10^7	5×10^5 - 5×10^7

Table 2: Molar mass separation ranges for TOYOPEARL HW resins



Globular proteins

* TOYOPEARL HW-55 is base material for some IEC and HIC products

** TOYOPEARL HW-65 is base material for most IEC, HIC, and AFC products

Calibration molecules

Dextrans

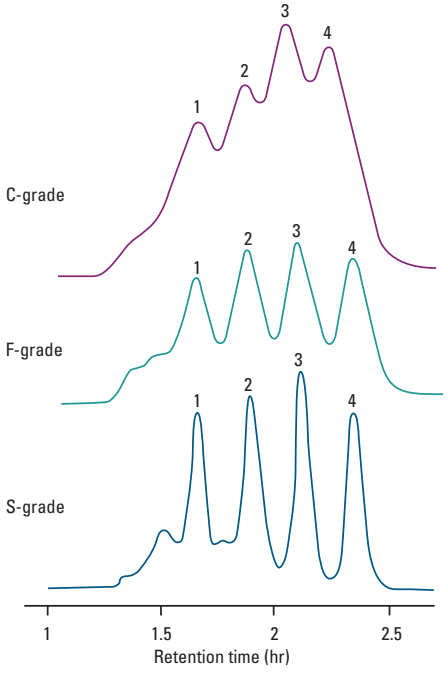
Polyethylene glycols and oxides

Resolution increases with decreasing particle size (Figure 3). Resin particle size is proportional to HETP and inversely proportional to the column efficiency and resolution of two peaks. Most TOYOPEARL HW resins are available in three particle size ranges:

- S-grade = 20 - 40 µm (Superfine)
- F-grade = 30 - 60 µm (Fine)
- C-grade = 50 - 100 µm (Coarse)

When the highest resolution is needed, the smaller S and F grade beads are preferred for process SEC. For desalting, where the resin is used in a filtration mode to remove the target from a buffer, the C grade is primarily employed because of its better flow dynamics at lower operating pressures. TOYOPEARL HW-40 is manufactured in an "EC-grade" (extra coarse) with a 100 - 300 µm bead.

Figure 3: Comparison of resolution on different particle sizes of TOYOPEARL HW-55 resin



Resin:	**TOYOPEARL HW-55**
Column size:	26 mm ID × 70 cm
Mobile phase:	33.3 mmol/L phosphate buffer, pH 7.0, 0.2 mol/L NaCl
Flow rate:	20 cm/h (1.77 mL/min)
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	1 mL
Samples:	1. thyroglobulin (0.3 %) 2. γ-globulin (0.3 %) 3. β-lactoglobulin (0.3 %) 4. cytochrome C (0.1 %)

General properties of TOYOPEARL HW resins in aqueous eluents are detailed in Table 3. TOYOPEARL HW resins can be used in organic solvents or mixtures of organic solvents and water. Bed volumes may swell or shrink relative to water depending on the solvent as shown in Tables 4 and 5. DMSO can be used for SEC of oligosaccharides and polyethylene glycols. The compatibility of DMF with TOYOPEARL also permits SEC separation of hydrophobic substances such as polystyrenes.

Table 3: Properties of TOYOPEARL HW resins in aqueous eluents

High mechanical stability	All TOYOPEARL resins can be operated at pressures up to 3 bar without deformation.
Minimum change in gel bed volume	Changes in the column bed volume under operational salt conditions are negligible. TOYOPEARL does not shrink or swell even in high concentrations of strong denaturing agents such as urea or guanidine hydrochloride.
Chemical stability	TOYOPEARL is stable from pH 2-13 and can tolerate levels outside of that range (pH 0-14) for short periods of time. Biomolecules which are only soluble at extreme pH values can be readily separated.
Sharp chromatographic peaks	TOYOPEARL's narrow particle size distribution (min. 80 % – within declared limits) results in better peak shapes and higher elution target concentrations than other SEC materials.
Temperature stability	TOYOPEARL HW SEC resins are thermally stable and do not degrade or denature even in boiling water. TOYOPEARL resins can be sterilized by autoclaving at 121 °C.
Microorganism resistance	TOYOPEARL is an organosynthetic material and is resistant to degradation by microorganisms.
Suitability for enzyme immobilization	TOYOPEARL resins contain numerous hydroxyl groups on the external and internal bead surfaces. These, in combination with the chemical stability of the polymer, make the resin well suited for the covalent bonding of enzymes or other ligands. Please see the affinity chromatography section for more information.

Table 4: Swelling properties in various solvents

Mobile phase	TOYOPEARL resin				
	HW-40	HW-50	HW-55	HW-65	HW-75
Water	100	100	100	100	100
0.2 mol/L KCl	100	100	100	100	100
MeOH	100	100	100	100	105
EtOH	100	100	100	100	110
DMF	110	110	105	105	120
Acetone	80	80	85	90	110
Toluene	65	70	70	75	90

Table 5: Additional swelling data for TOYOPEARL HW-40 resin

TOYOPEARL resin	Mobile phase				
	DMSO	Ethyl Acetate	Benzene	$CHCl_3$	$CHCl_3$/ MeOH (1:1)
HW-40	140	80	70	105	120

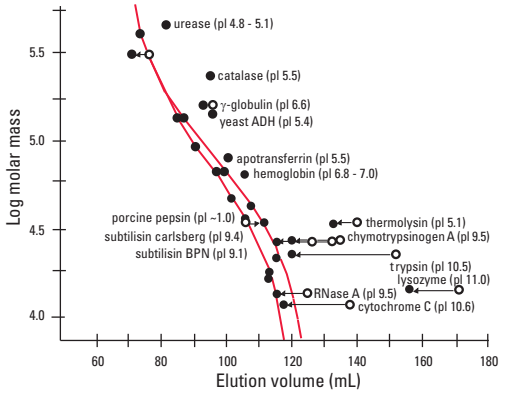
Mobile phase components, such as salts, can affect SEC separations. The presence or absence of sodium chloride influences the elution volume of proteins. This is demonstrated in Figure 4, in which a mixture of various proteins was separated on a column packed with TOYOPEARL HW-55F resin. Salt concentrations can change the hydrodynamic radius of proteins and either increase or decrease their molar mass as a function of salt strength.

TOYOPEARL HW resins are commonly used in size exclusion chromatography and desalting applications though they can be used for other important functions, such as:

- Removal of surfactants such as Triton® X-100 from biological solutions by an adsorption mechanism
- Use in hydrophobic interaction chromatography (HIC) for the separation of very hydrophobic molecules
- Use in HIC separations as a guard column for hydrophobic impurities
- Possible use as a stationary phase for either normal or reversed phase separations depending on solvent system selected

All of the physical and chemical properties discussed for the TOYOPEARL HW SEC resins make them an excellent choice for use as the base beads for the ion exchange, hydrophobic interaction, mixed-mode, and affinity chromatographic resins discussed in the later sections of this catalog.

Figure 4: Comparison of the elution volumes of proteins in presence and absence of NaCl

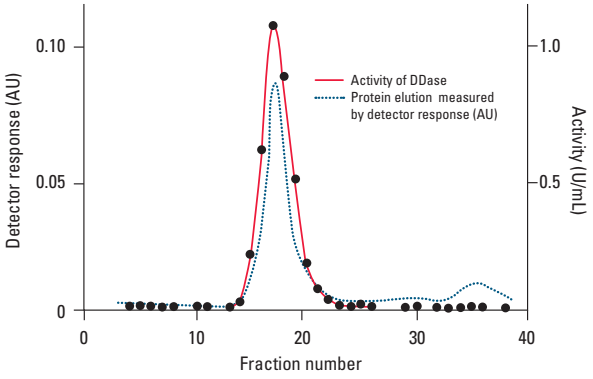


Resin:	**TOYOPEARL HW-55F**
Column size:	22 mm ID × 50 cm
Mobile phase:	25 mmol/L Tris-HCl buffer with (●) or without (○) 0.5 mol/L NaCl, pH 7.5
Flow rate:	16 cm/h (1.0 mL/min)
Detection:	UV @ 280 nm, 420 nm for heme proteins, 200 nm for proteins without aromatic amino acids
Temperature:	5-10 °C

Polishing Step for Enzyme Purification

TOYOPEARL HW SEC resins are an excellent choice when looking for a polishing step for enzyme purification. Dextrin dextranase, a 300 kDa enzyme, was purified from *Acetobacter capsulatus* using a two-step process consisting of TOYOPEARL Phenyl-650M and TOYOPEARL HW-65S. The elution profile of Dextrin dextranase is shown in (Figure 5). Due to the hydrophobic nature of the enzyme, it aggregates in 100 % aqueous mobile phases, thus it was necessary to add 40 % ethylene glycol to the mobile phase.

Figure 5: Elution profile of protein and activity on TOYOPEARL HW-65S

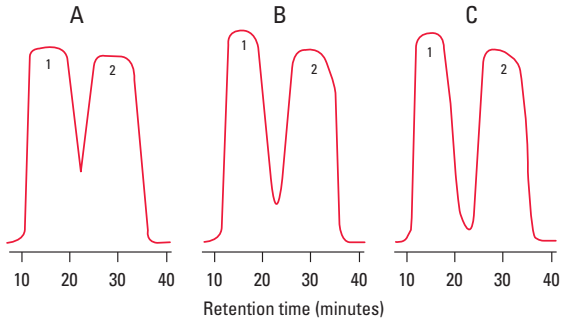


Resin:	**TOYOPEARL HW-65S**
Column size:	16 mm ID × 80 cm
Mobile phase:	20 mol/L acetate buffer with 40 % ethylene glycol, pH 4.8
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	concentrated active fractions from TOYOPEARL Phenyl-650 column

Desalting Step for Proteins

Though SEC is typically used as the polishing step in a purification process, it can also be used as an ideal desalting step for proteins that may be sensitive to membrane concentration and diafiltration steps. TOYOPEARL HW-40F allows for high total protein and activity recovery, allowing the operator to use it as a desalting resin. Figure 6 demonstrates the effect of the ionic strength of a volatile salt on the desalting of bovine serum albumin (BSA) from sodium acetate. It is important to note that the loading volumes for a desalting application are much higher than for regular SEC purifications. As much as 25 % of the bed volume can be loaded for desalting steps, compared with 1 % to 5 % of the bed volume for normal SEC purifications.

Figure 6: Desalting of bovine serum albumin

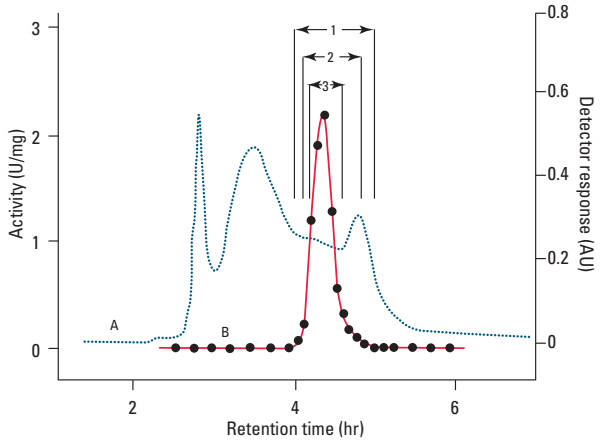


Resin:	**TOYOPEARL HW-40F**
Column size:	7.5 mm ID× 30 cm
Mobile phase:	A. distilled water B. 10 mmol/L ammonium formate C. 50 mmol/L ammonium formate
Flow rate:	48 cm/h (0.4 mL/min)
Detection:	UV @ 220 nm
Temperature:	25 °C
Samples:	2.5 mL of: 1. bovine serum albumin (0.1 %) 2. sodium acetate (10 %)

Recovery of Activity

Recovery of activity is a very important consideration when purifying an enzyme. As shown in Figure 7, crude β-galactosidase has been purified using TOYOPEARL HW-55F with excellent recovery yields (Table 6).

Figure 7: Purification of crude β-galactosidase on TOYOPEARL HW-55F



Resin:	**TOYOPEARL HW-55F**
Column size:	25 mm ID × 55 cm × 2
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.7
Flow rate:	12 cm/h (1.0 mL/min)
Detection:	A. UV @ 280 nm B. enzymatic activity
Temperature:	22 °C
Sample:	3 mL containing 45 mg of crude β-galactosidase

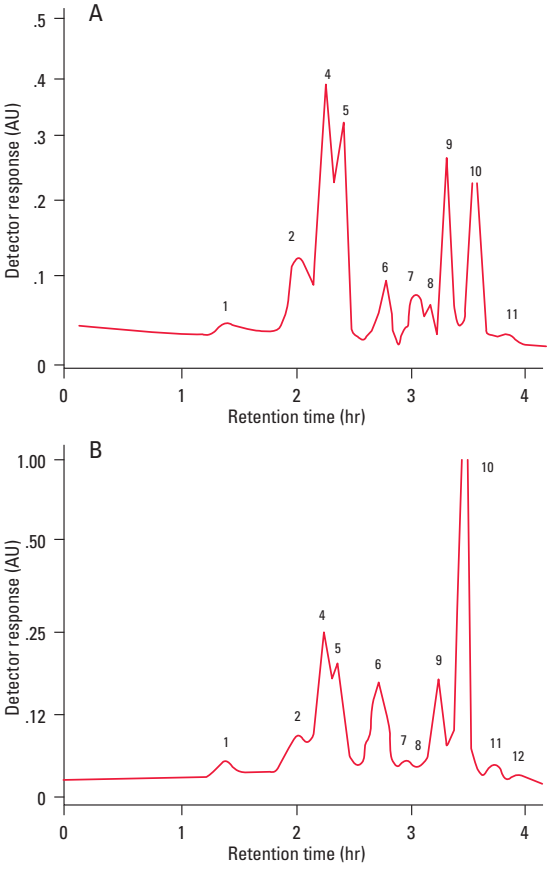
Table 6: Purification of crude β-galactosidase on TOYOPEARL HW-55F

Fraction	Yield (%)	Specific activity (units/mg)	Degree of purification
Original sample		0.95	
1	94	2.8	2.9 ×
2	93	3.7	3.9 ×
3	83	6.4	6.7 ×

Antibody Separation

Antibodies have been separated from bovine colostral whey and human serum using TOYOPEARL HW-55F resin. Figure 8 shows the separation of colostral whey on TOYOPEARL HW-55F after centrifugation. Peak #2 is IgG_1, and the chromatogram shows both the 254 and 280 nm absorbance profiles.

Figure 8: Elution profiles of colostral whey

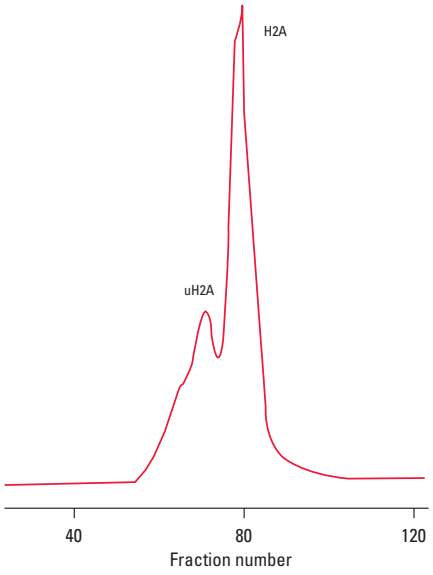


Resin:	**TOYOPEARL HW-55F**
Column size:	17 mm ID × 64 cm
Mobile phase:	25 mmol/L Tris-HCl buffer with 0.3 mol/L NaCl, pH 7.5
Flow rate:	18 cm/h (0.7 mL/min)
Detection:	A. UV @ 280 nm B. UV @ 254 nm
Temperature:	23 °C
Samples:	1. unknown 2. IgG_1 3. serum albumin 4. β-lactoglobulin (dimer) 5. α-lactalbumin 6 - 12. unknown

Isolation Based on Polypeptide Difference

TOYOPEARL HW-50S can help to isolate the ubiquitin-histone conjugate uH2A from the unicellular protozoan *Tetrahymena pyriformis*. Figure 9 shows the separation of uH2A from the histone, H2A. The sole difference between these two components is a small polypeptide, ubiquitin (approximately 8,500 Da).

Figure 9: Isolation of a complex protein conjugate on TOYOPEARL HW-50S

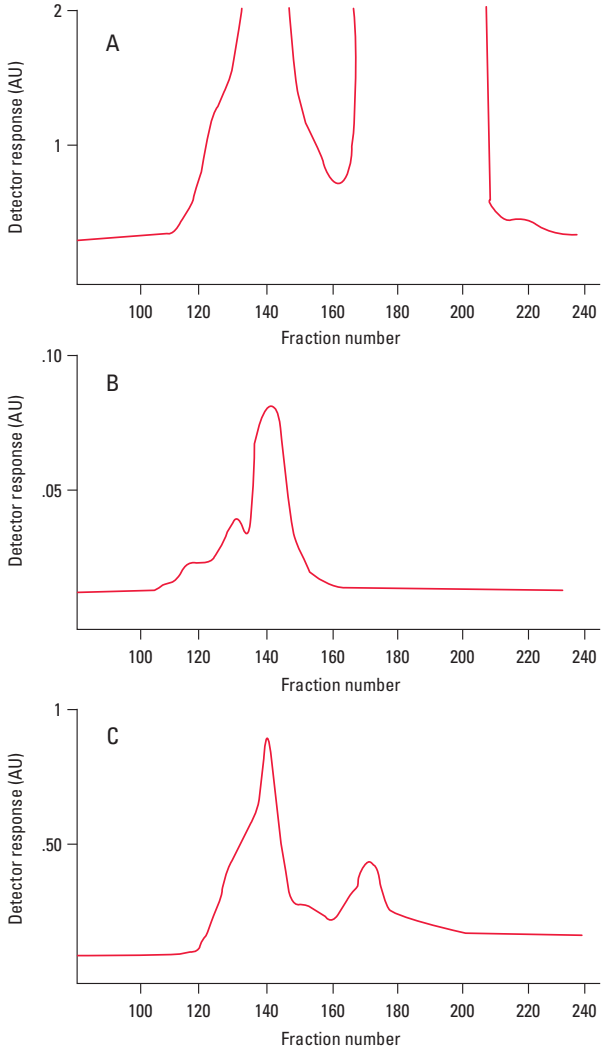


Resin:	**TOYOPEARL HW-50S**
Column size:	22 mm ID × 83 cm
Mobile phase:	10 mmol/L HCl
Flow rate:	1.6 cm/h (0.1 mL/min)
Detection:	UV @ 230 nm
Sample:	fraction of crude *Tetrahymena* H2A containing the ubiquitin-histone conjugate uH2A

Amylose Isolation

TOYOPEARL HW-75F resin, with pores larger than 100 nm, has been used in place of ultra-centrifugation steps for purification of plasmid DNA. Ultra-centrifugation is a time-consuming process and requires expensive chemicals, such as cesium chloride. TOYOPEARL HW-75F resin provides superior separation performance for plasmid DNA, and also provides high yields. Figure 10 shows the separation of crude pBR322 DNA from contaminating RNA species using TOYOPEARL HW-75F.

Figure 10: Separation of pBR322 DNA



Resin:	**TOYOPEARL HW-75F**
Column size:	16 mm ID × 130 cm
Mobile phase:	10 mmol/L Tris-HCl buffer, 1 mmol/L EDTA, 0.2 mol/L NaCl, pH 8
Flow rate:	7 cm/h (0.23 mL/min)
Detection:	UV @ 260 nm
Temperature:	ambient
Samples:	A. crude plasmid DNA extract B. DNA from A C. DNA from hydroxyapatite chromatography

A selection of screening tools are available for TOYOPEARL HW resins. See the Process Development Products section of this Product Guide for details.

Ordering Information

TOYOPEARL SEC resins:

Conditions: Exclusion limits are +/- 30 % and are determined using PEG, PEO, or dextran standards, as appropriate.

Part #	Product description	Container size (mL)	Bead diameter (µm)	Exclusion limit (Da)
0019809	TOYOPEARL HW-40S	150	20-40	3×10^3
0007451	TOYOPEARL HW-40S	250		
0007447	TOYOPEARL HW-40S	500		
0014681	TOYOPEARL HW-40S	1,000		
0007967	TOYOPEARL HW-40S	5,000		
0019808	TOYOPEARL HW-40F	150	30-60	3×10^3
0007448	TOYOPEARL HW-40F	500		
0014682	TOYOPEARL HW-40F	1,000		
0007968	TOYOPEARL HW-40F	5,000		
0019807	TOYOPEARL HW-40C	150	50-100	3×10^3
0007449	TOYOPEARL HW-40C	500		
0014683	TOYOPEARL HW-40C	1,000		
0007969	TOYOPEARL HW-40C	5,000		
0021484	TOYOPEARL HW-40C	50,000		
0007450	TOYOPEARL HW-40EC	500	100-300	3×10^3
0007970	TOYOPEARL HW-40EC	5,000		
0019811	TOYOPEARL HW-50S	150	20-40	1.8×10^4
0007455	TOYOPEARL HW-50S	250		
0007452	TOYOPEARL HW-50S	500		
0014684	TOYOPEARL HW-50S	1,000		
0008059	TOYOPEARL HW-50S	5,000		
0019810	TOYOPEARL HW-50F	150	30-60	1.8×10^4
0007453	TOYOPEARL HW-50F	500		
0014685	TOYOPEARL HW-50F	1,000		
0008060	TOYOPEARL HW-50F	5,000		
0018368	TOYOPEARL HW-50F	50,000		
0019813	TOYOPEARL HW-55S	150	20-40	1.5×10^5
0007459	TOYOPEARL HW-55S	250		
0007456	TOYOPEARL HW-55S	500		
0014686	TOYOPEARL HW-55S	1,000		
0008062	TOYOPEARL HW-55S	5,000		
0019812	TOYOPEARL HW-55F	150	30-60	1.5×10^5

Part #	Product description	Container size (mL)	Bead diameter (µm)	Exclusion limit (Da)
0007457	TOYOPEARL HW-55F	500	30-60	1.5×10^5
0014687	TOYOPEARL HW-55F	1,000		
0008063	TOYOPEARL HW-55F	5,000		
0021918	TOYOPEARL HW-55F	50,000		
0019815	TOYOPEARL HW-65S	150	20-40	1×10^6
0007467	TOYOPEARL HW-65S	250		
0007464	TOYOPEARL HW-65S	500		
0014688	TOYOPEARL HW-65S	1,000		
0008068	TOYOPEARL HW-65S	5,000		
0018377	TOYOPEARL HW-65S	50,000		
0019814	TOYOPEARL HW-65F	150	30-60	1×10^6
0007465	TOYOPEARL HW-65F	500		
0014689	TOYOPEARL HW-65F	1,000		
0008069	TOYOPEARL HW-65F	5,000		
0021852	TOYOPEARL HW-65F	50,000		
0021481	TOYOPEARL HW-65C	150	50-100	1×10^6
0007466	TOYOPEARL HW-65C	500		
0014690	TOYOPEARL HW-65C	1,000		
0008070	TOYOPEARL HW-65C	5,000		
0021482	TOYOPEARL HW-65C	50,000		
0007471	TOYOPEARL HW-75S	250	20-40	8.25×10^6
0007468	TOYOPEARL HW-75S	500		
0008071	TOYOPEARL HW-75S	5,000		
0019816	TOYOPEARL HW-75F	150	30-60	8.25×10^6
0007469	TOYOPEARL HW-75F	500		
0014691	TOYOPEARL HW-75F	1,000		
0008072	TOYOPEARL HW-75F	5,000		

Anion Exchange Resins	Cation Exchange Resins
TOYOPEARL DEAE-650C	TOYOPEARL MegaCap® II SP-550EC
TOYOPEARL Q-600C AR	TOYOPEARL CM-650C
TOYOPEARL QAE-550C	TOYOPEARL SP-550C
TOYOPEARL SuperQ-650C	TOYOPEARL SP-650C
TOYOPEARL GigaCap® DEAE-650M	TOYOPEARL GigaCap CM-650M
TOYOPEARL GigaCap Q-650M	TOYOPEARL GigaCap S-650M
TOYOPEARL DEAE-650M	TOYOPEARL CM-650M
TOYOPEARL SuperQ-650M	TOYOPEARL SP-650M
TOYOPEARL DEAE-650S	TOYOPEARL CM-650S
TOYOPEARL GigaCap Q-650S	TOYOPEARL GigaCap S-650S
TOYOPEARL SuperQ-650S	TOYOPEARL SP-650S
TSKgel DEAE-5PW (30)	TSKgel SP-3PW (30)
TSKgel SuperQ-5PW (30)	TSKgel SP-5PW (30)
TSKgel DEAE-5PW (20)	TSKgel SP-5PW (20)
TSKgel SuperQ-5PW (20)	

The role of Ion Exchange Chromatography in Process Purification

Ion Exchange Chromatography (IEX) plays a major role in the large scale purification of biomolecules. Today, IEX is one of the most commonly used techniques for the purification of proteins, nucleic acids, peptides, and other biomolecules. IEX can be further separated into anion (AEX) and cation (CEX) exchange techniques, both offering high resolution separations with high loading capacities. Ion exchange chromatography is capable of separating species that have minor differences in charges, for example two proteins differing by a single charged amino acid. These attributes make IEX ideally suited to be used at any point in the purification process including capture, intermediate purification, and polishing steps. The scalability of this technique allows it to be used from discovery and analysis through to commercial manufacturing operations.

Ion exchange chromatography functions by separating molecules on the basis of charge differences. Molecules are diverse in their charge properties and interact with charged chromatography media based on differences in their charge density, net charge, and distribution of that charge across the surface of the molecule. Since all molecules with charged groups can be titrated, their net surface charge is largely pH dependent. The net surface charge of proteins, which contain many different amino acids of weakly acidic and basic groups, will change as the environmental pH of the proteins change. IEX chromatography takes advantage of the relationship between net surface charge and pH for each specific protein. In ion exchange chromatography, a reversible interaction between a charged molecule and an oppositely charged ligand are controlled to favor the binding or elution of specific molecules to achieve separation. A protein at the pH above its isoelectric point will bind to a positively charged medium (anion exchanger) and at a pH below its pI, a protein will bind to a negatively charged medium (cation exchanger). The ligand attached to a chromatographic resin determines the charge of an IEX medium, a positively-charged anion or a negatively-charged cation exchanger.

TOYOPEARL Ion Exchange Chromatography Resins

TOYOPEARL IEX resins are functionalized versions of the TOYOPEARL HW size exclusion resins and are therefore based on hydroxylated polymethacrylic polymer beads. Tosoh Bioscience offers four ligands for anion exchange (Q, SuperQ, QAE, and DEAE) and three ligands for cation exchange chromatography (S, SP, and CM). Table 1 lists the properties of these TOYOPEARL IEX resins.

Table 1: Properties of TOYOPEARL ion exchange resins

TOYOPEARL resins	Anion/Cation exchange	Base bead	Pore size	Bead diameter	Ligand type	Ligand pKa	DBC (g/L)	Pressure rating
SuperQ-650C	Anion	HW-65	100 nm	100 µm	Strong Anion	12.2	105 - 155	0.3 MPa
DEAE-650C	Anion	HW-65	100 nm	100 µm	Weak Anion	11.5	25 - 35	0.3 MPa
QAE-550C	Anion	HW-55	50 nm	100 µm	Strong Anion	12.2	60 - 80	0.3 MPa
Q-600C AR	Anion	HW-60	75 nm	100 µm	Strong Anion	12.2	> 120	0.3 MPa
GigaCap Q-650M	Anion	HW-65	100 nm	75 µm	Strong Anion	12.2	105 - 155	0.3 MPa
GigaCap DEAE-650M	Anion	HW-65	100 nm	75 µm	Weak Anion	11.5	> 156	0.3 MPa
SuperQ-650M	Anion	HW-65	100 nm	65 µm	Strong Anion	12.2	105 - 155	0.3 MPa
DEAE-650M	Anion	HW-65	100 nm	65 µm	Weak Anion	11.5	25 - 35	0.3 MPa
SuperQ-650S	Anion	HW-65	100 nm	35 µm	Strong Anion	12.2	105 - 155	0.3 MPa
DEAE-650S	Anion	HW-65	100 nm	35 µm	Weak Anion	11.5	25 - 35	0.3 MPa
GigaCap Q-650S	Anion	HW-65	100 nm	35 µm	Strong Anion	12.2	> 170	0.3 MPa
MegaCap II SP-550EC	Cation	HW-55	50 nm	200 µm	Strong Cation	1.2	60 - 90	0.3 MPa
SP-650C	Cation	HW-65	100 nm	100 µm	Strong Cation	1.2	35 - 55	0.3 MPa
SP-550C	Cation	HW-55	50 nm	100 µm	Strong Cation	1.2	80 - 120	0.3 MPa
CM-650C	Cation	HW-65	100 nm	100 µm	Weak Cation	4.7	25 - 45	0.3 MPa
GigaCap S-650M	Cation	HW-65	100 nm	75 µm	Strong Cation	1.2	80 - 120	0.3 MPa
GigaCap CM-650M	Cation	HW-65	100 nm	75 µm	Weak Cation	3.6	> 110	0.3 MPa
SP-650M	Cation	HW-65	100 nm	65 µm	Strong Cation	1.2	40 - 60	0.3 MPa
CM-650M	Cation	HW-65	100 nm	65 µm	Weak Cation	4.7	30 - 50	0.3 MPa
SP-650S	Cation	HW-65	100 nm	35 µm	Strong Cation	1.2	40 - 60	0.3 MPa
CM-650S	Cation	HW-65	100 nm	35 µm	Weak Cation	3.6	30 - 50	0.3 MPa
GigaCap S-650S	Cation	HW-65	100 nm	35 µm	Strong Cation	1.2	> 150	0.3 MPa

TSKgel Ion Exchange Chromatography Resins

The same SuperQ, DEAE, and SP ligands that are used for the TOYOPEARL resins are also available within the TSKgel IEX resin product line. The TSKgel IEX resins use the same methacrylic polymer chemistry as the TOYOPEARL resins but have a higher degree of crosslinking, making for a more rigid bead. This is necessitated by the higher pressures generated when using smaller particles for chromatography. Greater crosslinking decreases the number of sites available for ligand attachment and thus a TSKgel resin will have a lower dynamic binding capacity than the corresponding TOYOPEARL resin. The polymeric structure of these products also makes them resistant to a wide range of pH conditions and mobile phase ionic strengths. In addition, the hydroxylated surface of the base bead reduces non-specific binding of proteins. Table 2 lists the properties of these TSKgel IEX resins.

The semi-rigid backbone of both TOYOPEARL and TSKgel IEX resins permits high flow rates for maximum throughput and productivity. While TOYOPEARL IEX resins may be operated at pressures up to 0.3 MPa, TSKgel -5PW and -3PW resins may be operated up to 2.0 MPa. Depending on their bead size and the buffer system used, linear velocities of greater than 1,000 cm/h can be achieved.

Table 3 shows the ligands and particle sizes available for TOYOPEARL and TSKgel IEX resins and is arranged in increasing levels of resolution by bead size (i.e. low, medium, and high resolution). The availability of smaller bead sizes for greater resolution while maintaining the same selectivity is particularly useful in the areas of oligonucleotide and peptide purification.

Table 2: Properties of TSKgel ion exchange resins

TSKgel resins	Anion/Cation exchange	Base bead	Pore size	Bead diameter	Ligand type	Ligand pKa	DBC (g/L)	Pressure rating
DEAE-5PW (20)	Anion	G5000PW	100 nm	20 µm	Weak Anion	11.5	25 -45	2.0 MPa
DEAE-5PW (30)	Anion	G5000PW	100 nm	30 µm	Weak Anion	11.5	20 - 40	2.0 MPa
SuperQ-5PW (20)	Anion	G5000PW	100 nm	20 µm	Strong Anion	12.2	52 - 88	2.0 MPa
SuperQ-5PW (30)	Anion	G5000PW	100 nm	30 µm	Strong Anion	12.2	52 - 88	2.0 MPa
SP-3PW (30)	Cation	G3000PW	25 nm	30 µm	Strong Cation	1.2	> 65	2.0 MPa
SP-5PW (20)	Cation	G5000PW	100 nm	20 µm	Strong Cation	1.2	20 - 40	2.0 MPa
SP-5PW (30)	Cation	G5000PW	100 nm	30 µm	Strong Cation	1.2	20 - 40	2.0 MPa

Table 3: Resolution of TOYOPEARL and TSKgel ion exchange resins

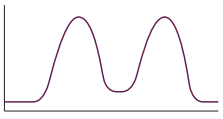
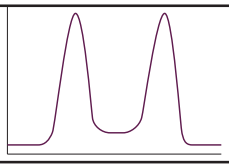
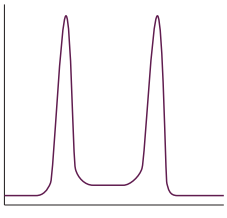
Resolution		Bead diameter (µm)	Pore size (nm)	Resins	
				Anion	Cation
Low		200	50		TOYOPEARL MegaCap II SP-550EC
		100	100 100 50	TOYOPEARL SuperQ-650C TOYOPEARL DEAE-650C TOYOPEARL QAE-550C	TOYOPEARL SP-650C TOYOPEARL CM-650C TOYOPEARL SP-550C
Medium		75	100 100	TOYOPEARL GigaCap Q-650M TOYOPEARL GigaCap DEAE-650M	TOYOPEARL GigaCap S-650M TOYOPEARL GigaCap CM-650M
		65	100 100 75	TOYOPEARL SuperQ-650M TOYOPEARL DEAE-650M TOYOPEARL Q-600C-AR	TOYOPEARL SP-650M TOYOPEARL CM-650M
High		35	100 100 100	TOYOPEARL SuperQ-650S TOYOPEARL DEAE-650S TOYOPEARL GigaCap Q-650S	TOYOPEARL SP-650S TOYOPEARL CM-650S TOYOPEARL GigaCap S-650S
		30	100 100 200	TSKgel SuperQ-5PW (30) TSKgel DEAE-5PW (30)	TSKgel SP-5PW (30) TSKgel SP-3PW (30)
		20	100 100	TSKgel SuperQ-5PW (20) TSKgel DEAE-5PW (20)	TSKgel SP-5PW (20)

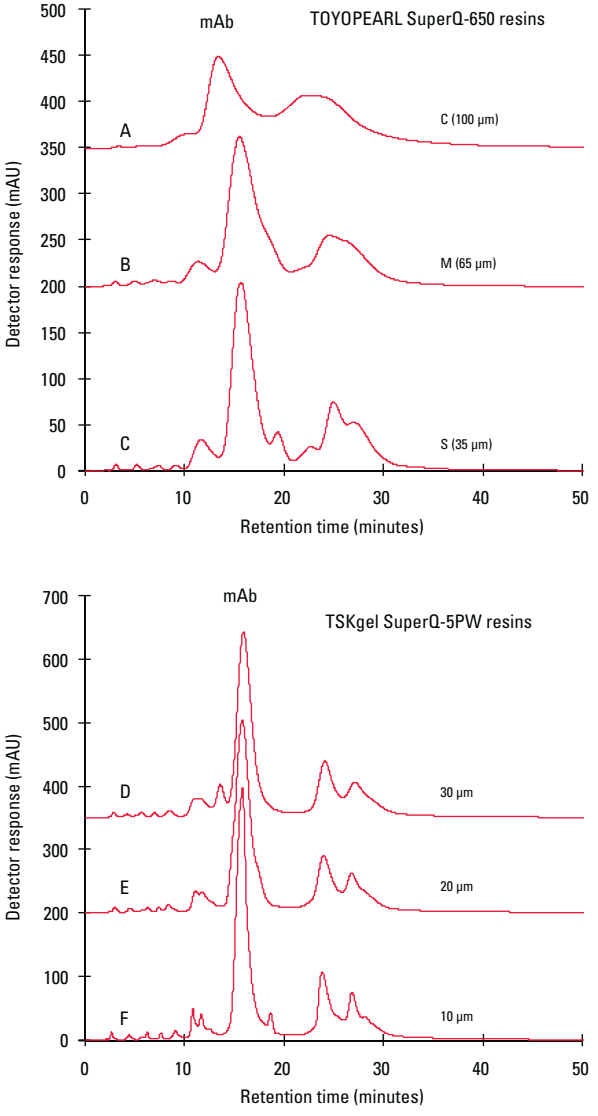
Table 4: DBCs of different chromatography modes

Separation mode	Binding capacity for standard proteins (g/L)	Binding capacity in production processes (g/L)
Ion Exchange	**100 - 200**	**50 - 100**
Hydrophobic Interaction	40 - 60	10 - 30
Affinity (group specific ligands)	40 - 100	20 - 60
Reversed Phase (polymeric media)	60 - 100	30 - 50

Due to the high dynamic binding capacities of ion exchange resins relative to those of the other chromatographic modes (Table 4), IEX is the chromatographic technique selected by many developers for the capture or concentration step.

Because TOYOPEARL and TSKgel IEX resins have the same backbone polymer chemistry, the selectivity for proteins and impurities will be unchanged. Due to this continuity between the TOYOPEARL and TSKgel resins, the chromatographic conditions that work for one particle size will work for all particle sizes with a given ligand functionality. The elution order of the feedstock components will remain the same with increasing resolution as the particle size gets smaller (Figure 1).

Figure 1: Scale up or down using the same ligand

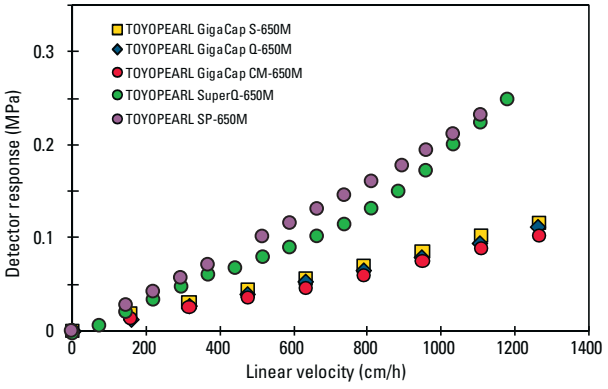


Resins:
A. TOYOPEARL SuperQ-650C, 100 µm
B. TOYOPEARL SuperQ-650M, 65 µm
C. TOYOPEARL SuperQ-650S, 35 µm
D. TSKgel SuperQ-5PW(30), 30 µm
E. TSKgel SuperQ-5PW(20), 20 µm
F. TSKgel SuperQ-5PW, 10 µm

Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 0.02 mol/L Tris-HCl buffer, pH 8.5
Buffer B: 0.5 mol/L NaCl in buffer A
Gradient: 60 min linear gradient from buffer A to buffer B
Flow rate: 136 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Sample: mAb in mouse ascites (dilution, x 5)
Load volume: 100 µL

The TOYOPEARL GigaCap M-grade resins have a particle size of 50-100 µm, which is slightly larger than the normal TOYOPEARL M-grade, 40-90 µm beads. This particle size difference generates a lower back pressure (Figure 2) than the more traditional TOYOPEARL M-grade ion exchange products. The TOYOPEARL GigaCap M-grade resins are high throughput resins that can be used for capture, intermediate, and polishing chromatographic steps.

Figure 2: Pressure-flow curve comparison of TOYOPEARL resins



Resins:	**TOYOPEARL GigaCap S-650M** **TOYOPEARL GigaCap Q-650M** **TOYOPEARL GigaCap CM-650M** **TOYOPEARL SuperQ-650M** **TOYOPEARL SP-650M**
Column size:	22 mm ID × 20 cm
Mobile phase:	distilled water
Detection:	pressure (MPa)
Temperature:	25 °C

TOYOPEARL and TSKgel IEX resins are chemically stable from pH 3-13. This allows a constant packing volume over a wide range of salt concentrations and cleaning in place (CIP) with acid or base. Also, these resins can be run at elevated temperatures (4-60 °C) and are autoclavable at 121 °C. Tosoh has focused on improving the alkaline stability of its newer ion exchange resins. Higher capacity resins can bind not only more of the target molecule, but the impurities and isoforms as well. In some cases more rigorous cleaning agents like 0.5 mol/L NaOH and even 1.0 mol/L NaOH are needed to ensure proper resin regeneration. Naturally, the resins need to tolerate these more stringent conditions.

TOYOPEARL IEX resins are available in a broad range of base bead pore sizes (Table 5). Of these, four different mean pore diameters are used: 100 nm, 75 nm, 50 nm, and 20 nm (Table 6). The TSKgel IEX resins have a base bead pore size of 100 nm with the exception of TSKgel SP-3PW, which has a pore size of 25 nm. A bead with a small pore size has theoretically more surface area than the same size bead with a larger pore. Please refer to Table 2 in the SEC section of this catalog (page 5) for the molar mass range of biomolecules covered by each pore size. Figure 3 shows insulin binding capacity on six different pore size beads. As the pore size increases to the point where the insulin has greatest access to the internal surface area, the insulin capacity increases. However, there is a point of diminishing return. Because the absolute surface area decreases as the pores become larger, the insulin capacity decreases accordingly.

Table 5: Methacrylic base beads available for IEC

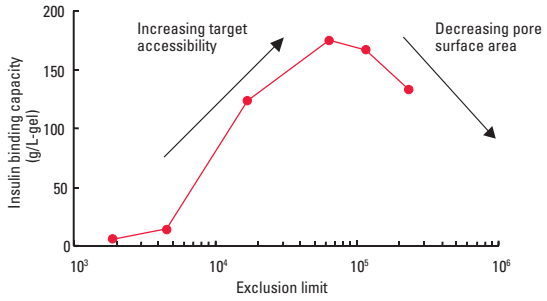
Pore size (nm)	5	12.5	40-50	75	100	>100	>170
Resin							
TOYOPEARL HW-type:	40	50	55	60	65	75	80
TSKgel PW-type:	G1000	G2000	G4000		G5000	G6000	

Increasing pore surface area

Table 6: Mean pore diameters used in TOYOPEARL and TSKgel IEX resins

Base bead	TOYOPEARL HW-65 or TSKgel G5000PW	TOYOPEARL HW-60	TOYOPEARL HW-55	TSKgelG3000PW
Pore diameter	**100 nm**	**75 nm**	**50 nm**	**25 nm**
Resin	TOYOPEARL GigaCap S-650 TOYOPEARL GigaCap CM-650 TOYOPEARL GigaCap Q-650 TOYOPEARL SuperQ-650 TOYOPEARL DEAE-650 TOYOPEARL SP-650 TOYOPEARL CM-650 TSKgel SuperQ-5PW TSKgel SP-5PW TSKgel DEAE-5PW	TOYOPEARL Q-600C AR	TOYOPEARL SP-550 TOYOPEARL MegaCap II SP-550 TOYOPEARL QAE-550	TSKgel SP-3PW

Figure 3: Optimization of insulin binding capacity as a function of pore size of experimental TSKgel SP-type resins



Additional modifications to ligand and bead chemistry resulted in the TOYOPEARL Q-600C AR (alkaline resistant) resin. This is a high capacity, alkaline resistant, Q anion exchange media. TOYOPEARL Q-600C AR resin (using first generation ligand attachment chemistry) was developed by Tosoh for CIP of difficult to remove impurities. This resin has a slightly smaller pore size than TOYOPEARL GigaCap Q-650M resin and has a typical BSA binding capacity of 100 g/L. As shown in Figure 4, after 100 days of exposure to 1.0 mol/L NaOH, the DBC of TOYOPEARL Q-600C AR resin remains unchanged. Figure 5 shows the preservation of selectivity after extensive exposure to caustic.

Figure 4: TOYOPEARL Q-600C AR resin DBC as a function of sodium hydroxide exposure

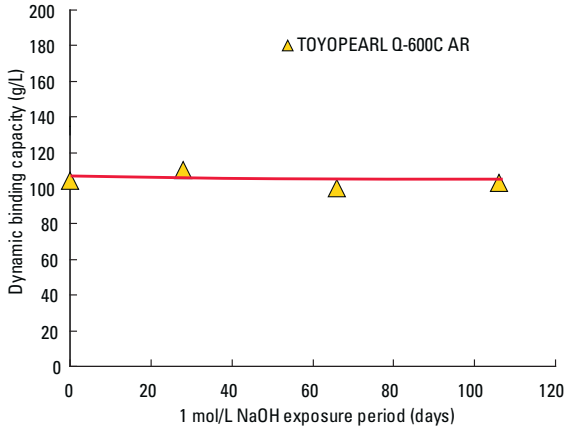
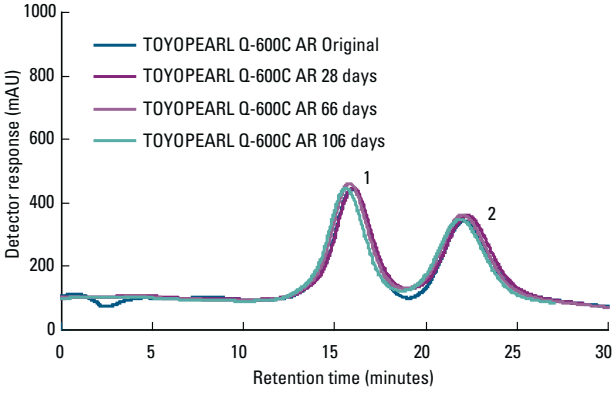


Figure 5: Stability of TOYOPEARL Q-600C AR resin after exposure to 1 mol/L NaOH



Resin:	**TOYOPEARL Q-600C AR**
Column size:	6.0 mm ID × 4 cm
Mobile phase:	Buffer A: 0.05 mol/L Tris-HCl buffer, pH 8.5
	Buffer B: 0.05 mol/L Tris-HCl buffer + 1.0 mol/L NaCl, pH 8.5
Gradient:	60 min linear gradient from buffer A to buffer B
Flow rate:	212 cm/h (1.0 mL/min)
Detection:	UV @ 280 nm
Samples:	1. ovalbumin
	2. soybean trypsin inhibitor

Following is an explanation of the three ligand attachment chemistries used by Tosoh for TOYOPEARL and TSKgel IEX resins:

Attachment type	TOYOPEARL resins	TSKgel resins
The "traditional" ligand attachment method consists of attaching the ion exchange ligand directly to the resin surface through a proprietary spacer arm.	SP-650 MegaCap II SP-550 EC SP-550 Q-550 DEAE-650 CM-650	SP-3PW SP-5PW DEAE-5PW
The second generation ligand attachment method, for the purpose of increasing protein binding within the accessible surface area, adds a carbon spacer network between the bead surface and the ligand. It is also possible to attach ligand groups along the length of the spacer network, thus improving capacity.	SuperQ-650	SuperQ-5PW
The third generation ligand attachment method improves the accessible location of the ligand groups. This ligand chemistry moves the charged groups to the larger pores where the protein has better access to them. The result of this modification is significantly increased capacity and improved mass transfer. Improved mass transfer also reduces the target molecule elution volume.	GigaCap Q-650 GigaCap CM-650 GigaCap S-650 GigaCap DEAE-650	

Table 7 contains DBC data for five TOYOPEARL resins using three different size proteins. There are three different pore sizes and three different ligand attachment methods represented. TOYOPEARL GigaCap Q-650M resin has the highest capacity for all combinations of pore size and attachment chemistries.

Table 7: DBC varies with protein size

Resin	Pore size (nm)	Binding capacity (g/L-gel)		
		BSA 66 kDA	Human IgG 160 kDA	Thyroglobulin 660 kDA
TOYOPEARL GigaCap Q-650M	100	173	108	71
TOYOPEARL SuperQ-650M	100	145	13	3
TOYOPEARL Q-600C AR	75	108	90	26
TOYOPEARL QAE-550C	50	29	32	6
TOYOPEARL DEAE-650M	100	25	31	3

Column size: 6.0 mm ID × 4 cm
Mobile phase: Buffer A:
BSA 0.05 mol/L Tris-HCl buffer, pH 8.5
Human IgG 0.05 mol/L Tris-HCl buffer, pH 8.7
Thyroglobulin 0.05 mol/L Tris-HCl buffer, pH 8.7 + 0.15 mol/L NaCl
Buffer B: 0.05 mol/L Tris-HCl buffer + 1.0 mol/L NaCl, pH 8.5
Flow rate: 212 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Samples: BSA, human IgG, thyroglobulin, each at 1.0 g/L

The following guidelines may be helpful when selecting a resin that is available in different pore sizes with the same ligand and ligand attachment chemistry:

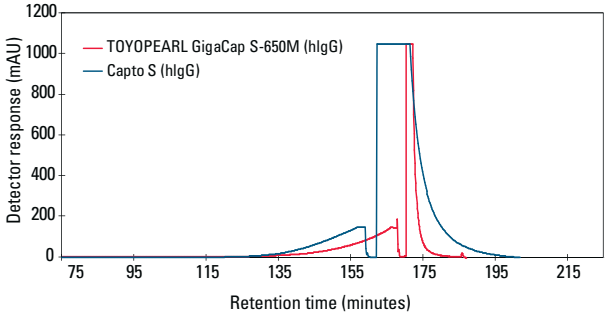
For bind/elute chromatography:	• Select the smallest pore size resin appropriate for the size of the target molecule. • Select a larger particle size for a capture step, a smaller one for intermediate or polishing steps.
For flow through chromatography:	• If the target molecule's size is larger than most components of the feed stream, select a pore size which will tend to exclude it (known as kinetic exclusion, this technique can also be used under binding conditions as the excluded molecule only sees 1 % of the resin surface area and the capacity/recovery loss is minimal).
For large molecule impurity clearance:	• Select a pore size which includes the target molecule, but excludes the impurity (see the calibration curves of the TOYOPEARL base beads in the SEC section of the catalog as an aid).

TOYOPEARL GigaCap Resins

TOYOPEARL GigaCap resins have both higher capacity and improved elution kinetics compared to corresponding TOYOPEARL IEX resins. When these parameters are combined, they may significantly reduce elution pool volumes by as much as 75 %. The TOYOPEARL GigaCap ligand attachment chemistry results in preferential placement of the functional groups into the larger more protein-accessible pores promoting both higher protein dynamic binding capacities and improved resin binding and desorption.

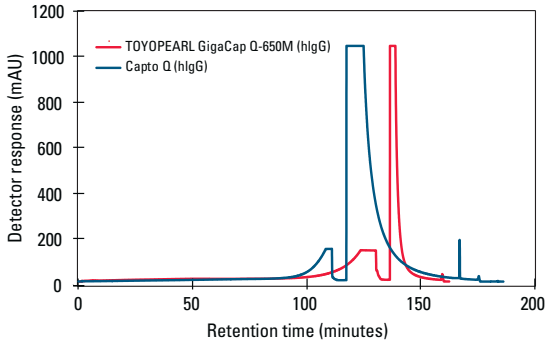
Unmodified TOYOPEARL HW-65 resin is utilized as the base bead for the TOYOPEARL GigaCap M-grade resins. The average particle size of the TOYOPEARL GigaCap M-grade resins, 75 µm, provides for enhanced efficiency and higher resolution than other larger particle size materials, while improved pressure-flow properties are obtained over smaller particle size materials. Figures 6, 7 and 8 show the breakthrough curves for three TOYOPEARL GigaCap M-grade resins. They are compared where possible with the most current equivalent competitive resin. Each trace shows the dynamic binding capacity of the resin up to 10 % breakthrough plus the elution profile for the target molecule. **Please note the significant reduction in elution pool volumes of the TOYOPEARL GigaCap resins when compared to other products. The concentration of the eluted peak is proportionally increased as well.**

Figure 6: Elution pool volume comparison of TOYOPEARL GigaCap S-650M vs. Capto™ S resins



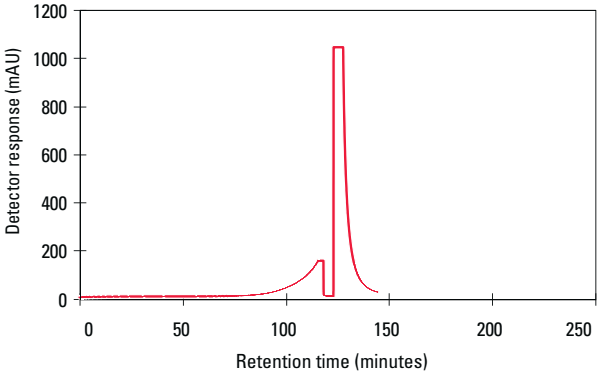
Resins:	**TOYOPEARL GigaCap S-650M**
	Capto S
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 0.1 mol/L acetate buffer, pH 4.7
	Buffer B: 0.1 mol/L acetate buffer, pH 4.7 + 1.0 mol/L NaCl
Flow rate:	212 cm/h (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	polyclonal human IgG (1 g/L)

Figure 7: Elution pool volume comparison of TOYOPEARL GigaCap Q-650M vs. Capto Q resins



Resins:	**TOYOPEARL GigaCap Q-650M**
	Capto Q
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 15 mmol/LTris-HCl buffer, pH 8.7
	Buffer B: 15 mmol/L Tris-HCl buffer, pH 8.7 + 1.0 mol/L NaCl
Flow rate:	212 cm/h (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	polyclonal human IgG (1 g/L)

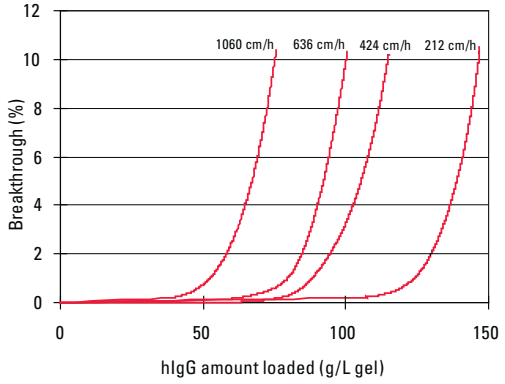
Figure 8: Elution pool volume of TOYOPEARL GigaCap CM-650M resin



Resin:	**TOYOPEARL GigaCap CM-650M**
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 50 mmol/L sodium acetate buffer, pH 4.7
	Buffer B: 50 mmol/L sodium acetate buffer, pH 4.7 + 0.5 mol/L NaCl
Flow rate:	212 cm/h (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	polyclonal human IgG (1 g/L)

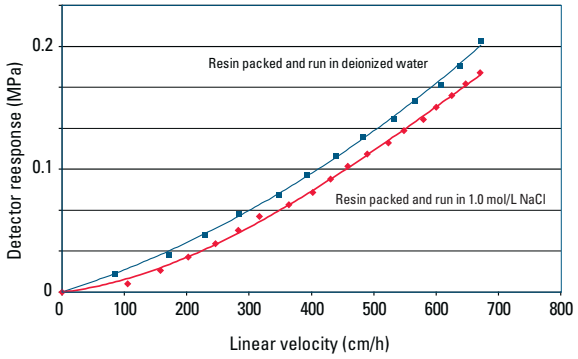
TOYOPEARL GigaCap S-650M resin was specifically developed for the purification of monoclonal antibodies. It has excellent elution kinetics (Figure 6) and maintains reasonably high capacities at higher linear velocities (Figure 9). The slightly larger particle size (50-100 µm) has been optimized to give a unique combination of improved pressure-flow characteristics (Figure 10) with excellent resolution at high loads (Figure 11). In separate studies it was established that DBC values for smaller proteins, such as insulin and lysozyme, were also notably improved with typical values of 133 g/L and 167 g/L, respectively.

Figure 9: TOYOPEARL GigaCap S-650M human IgG breakthrough curves at various linear velocities



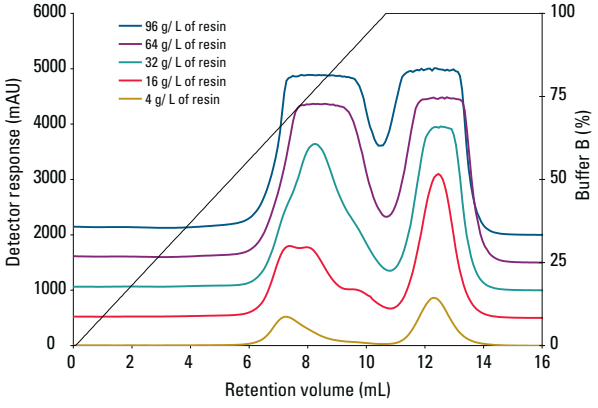
Resins:	**TOYOPEARL GigaCap S-650M**
Column size:	6 mm ID × 4 cm (1.13 mL)
Mobile phase:	0.1 mol/L acetate buffer, pH 4.7
Flow rates:	212, 424, 636, 1060 cm/h (1.0, 2.0, 3.0, 5.0 mL/min)
Detection:	UV @ 280 nm
Sample:	polyclonal human IgG (1 g/L)

Figure 10: Pressure flow data for TOYOPEARL GigaCap S-650M



TOYOPEARL GigaCap S-650M was packed into a 36 cm ID × 25 cm bed height Eastern Rivers BioStream column to measure the pressure-flow characteristics. The resin had similar profiles when packed and run in both water and 1.0 mol/L NaCl.

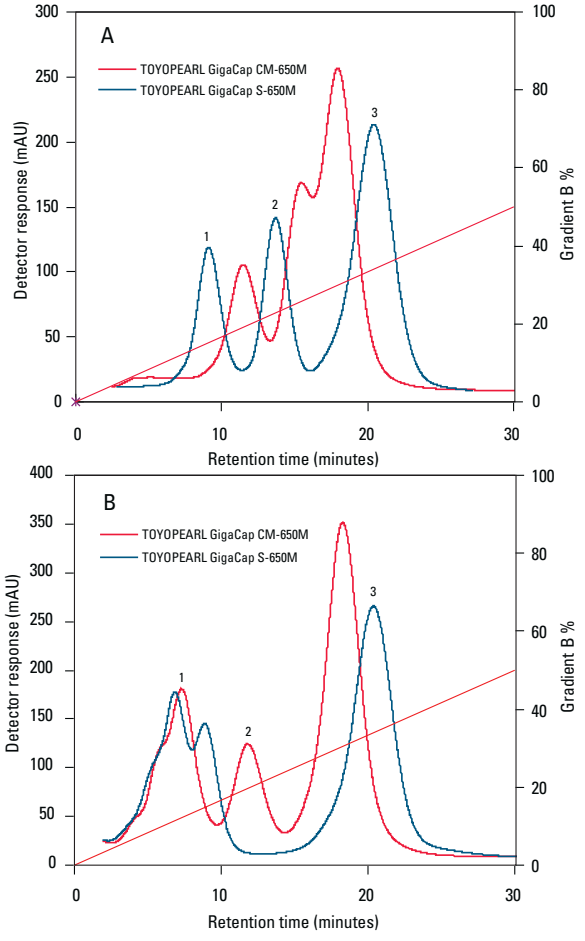
Figure 11: Resolution of proteins at high loading on TOYOPEARL GigaCap S-650M



Resin:	**TOYOPEARL GigaCap S-650M**
Column size:	3 mm ID × 15 cm
Mobile phase:	Buffer A: 20 mmol/L phosphate buffer, pH 6.0 Buffer B: 20 mmol/L phosphate buffer + 500 mmol/L NaCl, pH 6.0
Gradient:	10 CV linear gradient from 0 to 100 % B (0-500 mmol/L NaCl)
Flow rate:	300 cm/h (0.35 mL/min)
Detection:	UV @ 280 nm
Sample:	α-chymotrypsin (2 g/L), lysozyme (2 g/L) (total of 4 g proteins/L)

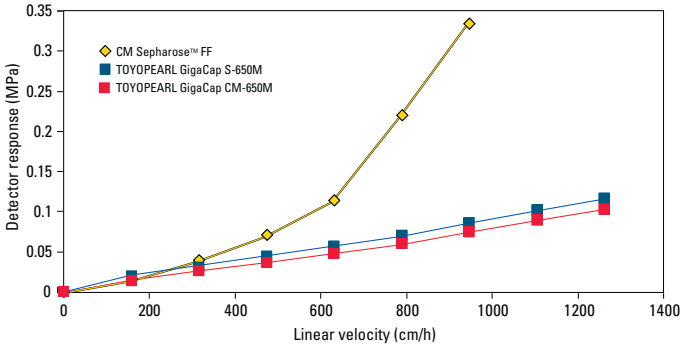
TOYOPEARL GigaCap CM-650M resin was designed for the purification of monoclonal antibodies that require a different chromatographic selectivity than is available with TOYOPEARL GigaCap S-650M resin (Figure 12). Excellent kinetic properties and high capacity are maintained at high linear flow velocities. Since TOYOPEARL GigaCap CM-650M resin is based on the same particle size base beads as the other resins within the TOYOPEARL GigaCap series, very good pressure-flow properties are obtained for this resin as well (Figure 13).

Figure 12: TOYOPEARL GigaCap CM-650M has unique selectivity



Resins:	**TOYOPEARL GigaCap CM-650M** **TOYOPEARL GigaCap S-650M**
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 20 mmol/L phosphate buffer, pH 7.0 Buffer B: 20 mmol/L phosphate buffer + 1.0 mol/L NaCl, pH 7.0
Gradient:	60 min linear gradient from buffer A to buffer B
Flow rate:	212 cm/h (1.0 mL/min)
Detection:	UV @ 280 nm
Injection vol.:	25 µL
Samples:	(A) 1. ribonuclease A (5.0 g/L) 2. cytochrome C (1.9 g/L) 3. lysozyme (3.8 g/L) (B) 1. trypsinogen (3.8 g/L) 2. ribonuclease A (5.0 g/L) 3. lysozyme (3.8 g/L)

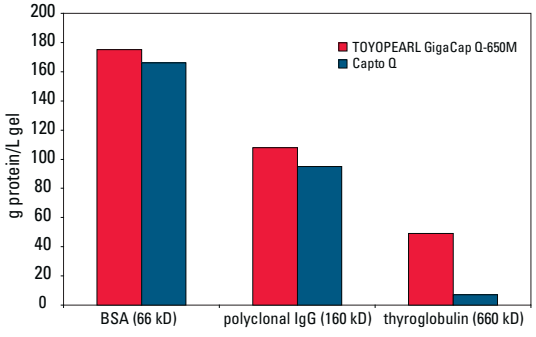
Figure 13: TOYOPEARL GigaCap CM-650M pressure-flow properties



Resin:	**TOYOPEARL GigaCap CM-650M**
Column size:	22 mm ID × 20 cm
Mobile phase:	water
Detection:	pressure (MPa)

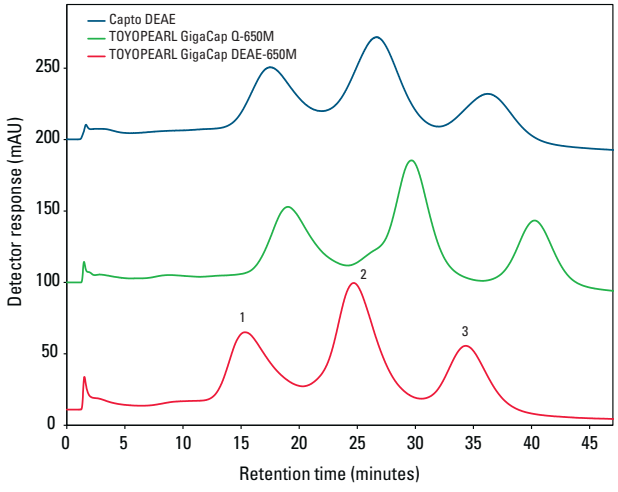
TOYOPEARL GigaCap Q-650M resin was primarily designed for the capture and purification of proteins, although it can also be used for polishing in flow-through chromatography. The particular note is the excellent capacity of TOYOPEARL GigaCap Q-650M for such large proteins as thyroglobulin when compared to other high capacity resins (Figure 14).

Figure 14: Dynamic binding capacity of proteins with different molar masses @ 212 cm/h



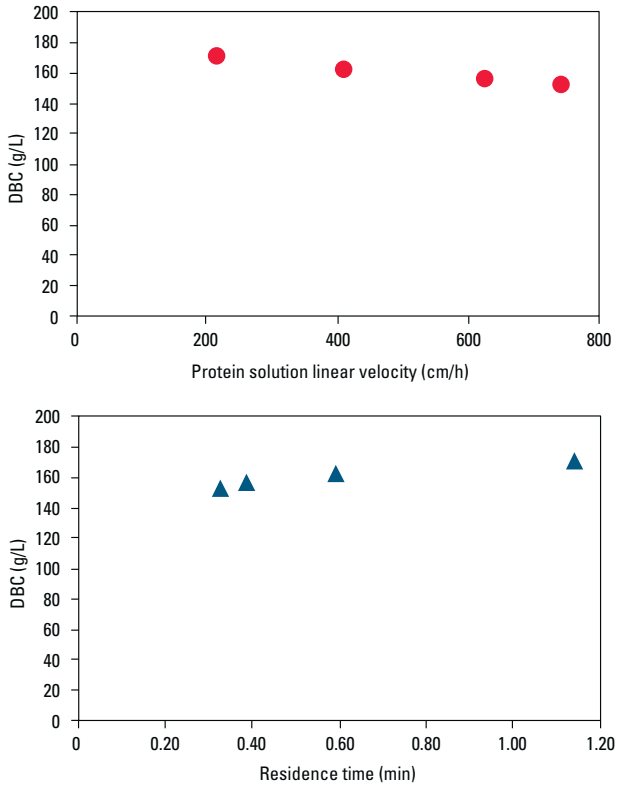
TOYOPEARL GigaCap DEAE-650M resin was designed for the purification proteins that require a different chromatographic selectivity (Figure 15) than is available with TOYOPEARL GigaCap Q-650M resin. As with other TOYOPEARL GigaCap M-grade resins, excellent kinetic properties and high capacity are maintained at high linear flow velocities (Figure 16). Since TOYOPEARL GigaCap DEAE-650M resin is based on the same particle size base beads as the other resins within the TOYOPEARL GigaCap series, very good pressure-flow properties are obtained for this resin as well (Figure 17).

Figure 15: Selectivity comparisons



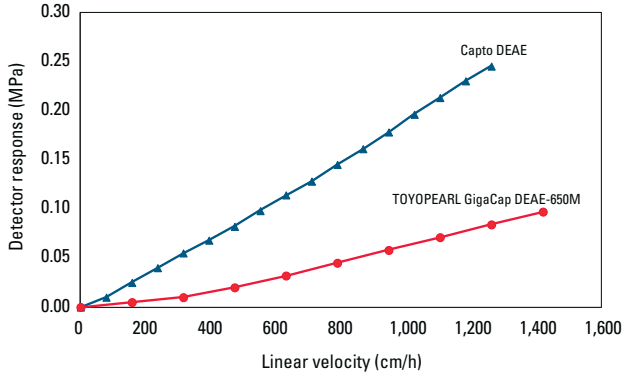
Resins:	**TOYOPEARL GigaCap DEAE-650M** **TOYOPEARL GigaCap Q-650M** Capto DEAE
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	Buffer A: 50 mmol/L Tris-HCl buffer, pH 8.5 Buffer B: buffer A + 1.0 mol/L NaCl, pH 8.5
Gradient:	120 minutes, 0 - 100 % B
Flow rate:	136 cm/h (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	100 µL
Samples:	1. transferrin, 2.9 g/L 2. ovalbumin, 6.5 g/L 3. trypsin inhibitor, 10.0 g/L
Sample load:	1.94 mg total protein

Figure 16: DBC vs. flow rate and residence time



Resins:	**TOYOPEARL GigaCap DEAE-650M**
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 0.05 mol/L Tris-HCl buffer, pH 8.5 Buffer B: mobile phase A + 1.0 mol/L NaCl
Flow rates:	212, 407, 623, and 739 cm/h (1.0, 1.9, 2.9, 3.5 mL/min)
Detection:	UV @ 280 nm
Sample:	BSA (1.0 g/L)

Figure 17: TOYOPEARL GigaCap DEAE-650M pressure-flow curves



Resin:	**TOYOPEARL GigaCap DEAE-650M**
Column size:	22 mm ID × 20 cm
Mobile phase:	0.1 mol/L NaCl
Detection:	pressure (MPa)

TOYOPEARL GigaCap Q-650 and S-650 resins are also available in a 35 µm S-grade, which is ideal for high resolution applications such as oligonucleotide, peptide and antibody-drug conjugate purifications. TOYOPEARL GigaCap Q-650S and TOYOPEARL GigaCap S-650S maintain the superior dynamic binding capacities (Tables 8 and 9) and selectivities (Figures 18 and 19) of the M-grade TOYOPEARL GigaCap resins with the benefit of greater resolution due to their smaller bead size. Pressure-flow properties (Figures 20 and 21) are also maintained with the TOYOPEARL GigaCap S-grade resins.

Table 8: Anion exchange resin binding capacity comparisons

Resin	Particle size (µm)	pH stability	Base bead	Ion exchange capacity (meq/L)	Binding capacity (g/L)		DBC recovery (%)	DBC elution volume (CV)
					Static	Dynamic*		
TOYOPEARL GigaCap Q-650S	20 - 50	3 - 13	polymethacrylic	0.20	200	191	99	1.7
TOYOPEARL GigaCap Q-650M	50 - 100	3 - 13	polymethacrylic	0.17	191	172	97	15.8
Capto™ Q ImpRes	36 - 44	2 - 12	agarose	0.12	92	40	100	ND**
Q Sepharose™ HP	24 - 44	2 - 12	agarose	0.15	114	81	99	ND**

*Dynamic binding capacities were determined at 10% breakthrough
**Values not determined

Dynamic Binding Capacity (DBC) Conditions:
Column size: 6 mm ID × 4 cm
Mobile phase: A: 50 mmol/L Tris-HCl buffer, pH 8.5
B: mobile phase A + 0.5 mol/L NaCl
Flow rate: 212 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Sample: 1.0 g/L BSA

Static Binding Capacity (SBC) Conditions:
Adsorption buffer: 50 mmol/L Tris-HCl buffer, pH 8.5
Protein concentration: 10.0 g/L

Table 9: Cation exchange resin binding capacity comparisons

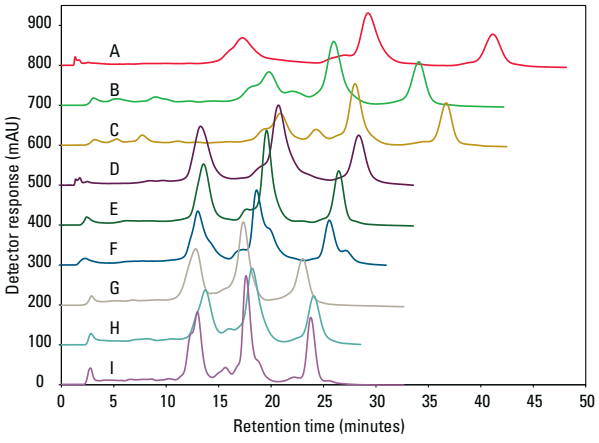
Resin	Particle size (µm)	pH stability	Base bead	Ion exchange capacity (meq/L)	Binding capacity (g/L)		DBC recovery (%)	DBC elution volume (CV)
					Static	Dynamic*		
TOYOPEARL GigaCap S-650S	20 - 50	3 - 13	polymethacrylic	0.24	177	164	99	4.0
TOYOPEARL GigaCap S-650M	50 - 100	3 - 13	polymethacrylic	0.16	156	145	98	13.5
Capto SP ImpRes	36 - 44	2 - 12	agarose	0.12	89	27	100	ND**
SP Sepharose™ HP	24 - 44	2 - 12	agarose	0.15	105	65	100	ND**

*Dynamic binding capacities were determined at 10% breakthrough
**Values not determined

Dynamic Binding Capacity (DBC) Conditions:
Column size: 6 mm ID × 4 cm
Mobile phase: A: 50 mmol/L acetate buffer, pH 4.7
B: mobile phase A + 0.5 mol/L NaCl
Flow rate: 212 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Sample: 1.0 g/L γ-globulin

Static Binding Capacity (SBC) Conditions:
Adsorption buffer: 50 mmol/L acetate buffer, pH 4.7
Sample: 10.0 g/L γ-globulin

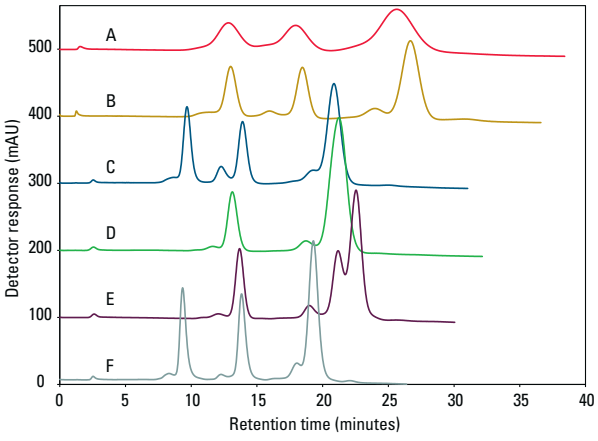
Figure 18: Selectivity comparisons of anion exchange resins



Resins:
A. TOYOPEARL GigaCap Q-650S
B. Capto Q ImpRes
C. Q Sepharose HP
D. TOYOPEARL SuperQ-650S
E. TSKgel SuperQ-5PW (30)
F. TSKgel SuperQ-5PW (20)
G. TOYOPEARL DEAE-650S
H. TSKgel DEAE-5PW (30)
I. TSKgel DEAE-5PW (20)

Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 50 mmol/L Tris-HCl buffer, pH 8.5
Buffer B: buffer A + 1.0 mol/L NaCl
Gradient: 0-100 % buffer B (120 min)
Flow rate: 136 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Injection vol.: 100 µL
Samples: transferrin, 2.9 g/L
ovalbumin, 6.5 g/L
trypsin inhibitor, 10.0 g/L

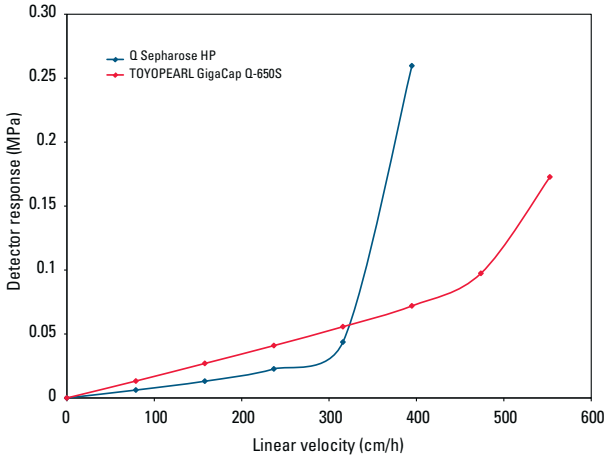
Figure 19: Selectivity comparisons of cation exchange resins



Resins:
A. TOYOPEARL GigaCap S-650M
B. TOYOPEARL GigaCap S-650S
C. TOYOPEARL SP-650S
D. Capto SP ImpRes
E. SP Sepharose HP
F. TSKgel SP-5PW (20)

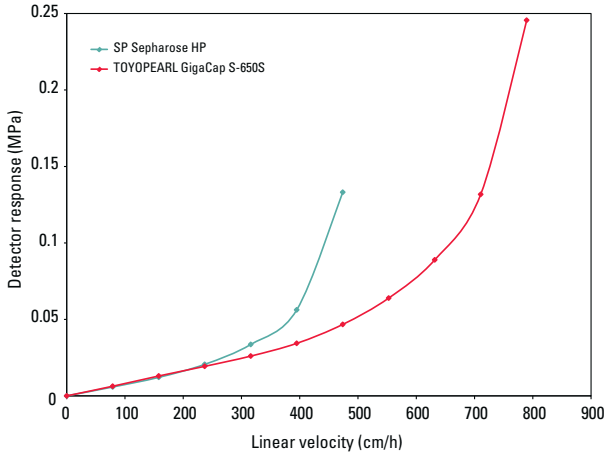
Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 20 mmol/L phosphate buffer, pH 7.0
Buffer B: buffer A + 1.0 mol/L NaCl
Gradient: 0-100 % buffer B (60 min)
Flow rate: 136 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Injection vol.: 20 µL
Samples: ribonuclease A, 9.8 g/L
cytochrome C, 3.6 g/L
lysozyme, 6.4 g/L

Figure 20: Comparison of TOYOPEARL GigaCap Q-650S and Q Sepharose HP pressure-flow curves



Resin: **TOYOPEARL GigaCap Q-650S**
Q Sepharose HP
Column size: 22 mm ID × 20 cm
Mobile phase: 0.1 mol/L NaCl
Detection: pressure (MPa)

Figure 21: Comparison of TOYOPEARL GigaCap S-650S and SP Sepharose HP pressure-flow curves



Resin: **TOYOPEARL GigaCap S-650S**
SP Sepharose HP
Column size: 22 mm ID × 20 cm
Mobile phase: 0.1 mol/L NaCl
Detection: pressure (MPa)

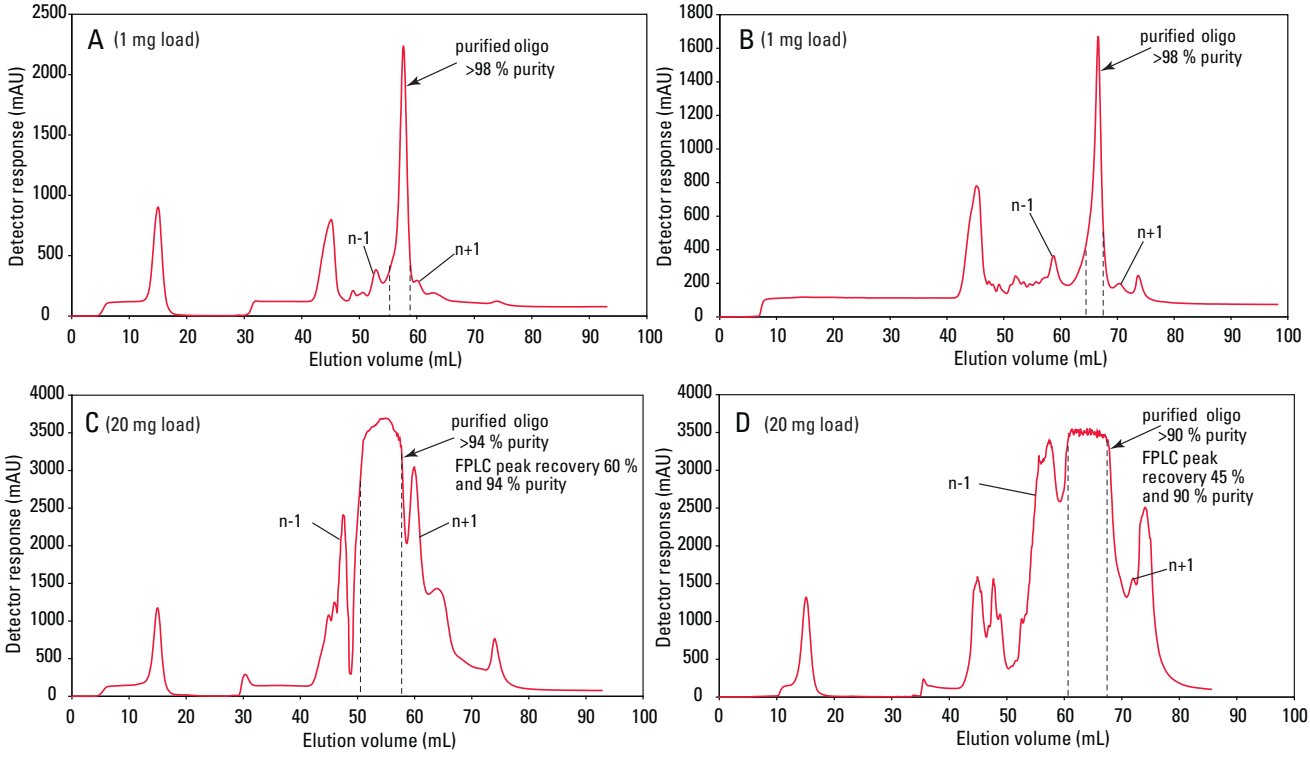
TSKgel SuperQ-5PW Resin

TSKgel SuperQ-5PW resin (offered in 20 and 30 µm particle size) is a strong anion exchange resin used for large and small biomolecules. TSKgel SuperQ-5PW analytical columns have the same backbone chemistry and selectivity as the bulk process scale TSKgel SuperQ-5PW resin, allowing seamless scale-up from analytical to manufacturing. In downstream processing of proteins, TSKgel SuperQ-5PW can be used for intermediate purification and polishing steps.

TSKgel SuperQ-5PW (20) resin is the product of choice for oligonucleotide purification. This resin does an excellent job as a capture resin isolating the full length oligonucleotide from the n-1, n+1, and other impurities generated during synthesis.

Figure 22 shows a comparison of one competitive product, of a smaller particle size, which initially has better resolution than TSKgel SuperQ-5PW (20) resin at 1 g oligonucleotide/L of resin. At 20 g oligonucleotide/L of resin, however, the resolution of peaks on the competitive product deteriorates significantly. The TSKgel SuperQ-5PW (20) resin retains excellent resolution even at this higher oligonucleotide concentration. Under higher loading conditions (Figure 22), the TSKgel SuperQ-type resins maintain their resolution much better than smaller particle, lower capacity resins. The smaller particle products may start out with a slight separation advantage under low oligonucleotide loading conditions, but this vanishes as the feedstock load is increased.

Figure 22: TSKgel SuperQ-5PW (20) resin maintains resolution at high oligonucleotide load



Resins:	**A & C: TSKgel SuperQ-5PW (20)**
	B & D: SOURCE™ 15Q
Column size:	0.66 cm × 15 cm (5.1 mL)
Mobile phase:	Buffer A: 20 mmol/L Tris-HCl buffer + 10 mmol/L EDTA, pH 9.0
	Buffer B: 20 mmol/L Tris-HCl buffer + 10 mmol/L EDTA + 1.0 mol/L NaCl, pH 9.0
Flow rate:	250 cm/h (1.43 mL/min)
Detection:	UV @ 254 nm
Sample:	DNA based oligonucleotides
Sample load:	A & B: 1 mg/column
	C & D: 20 mg/column
Separation conditions:	Column was washed with 5CV 100 % buffer A followed by 11 mL injection. Column was then washed with 3 CV 100 % buffer A followed by 6 CV of linear gradient 35-53 buffer B. Finally, column was washed with 5 CV 100 % buffer B.
Fractions:	0.5 mL fractions were taken from peaks of interest and analyzed on a TSKgel DNA-NPR column

Applications for Tosoh Bioscience Ion Exchange Chromatography Resins

Purification of Oligonucleotides

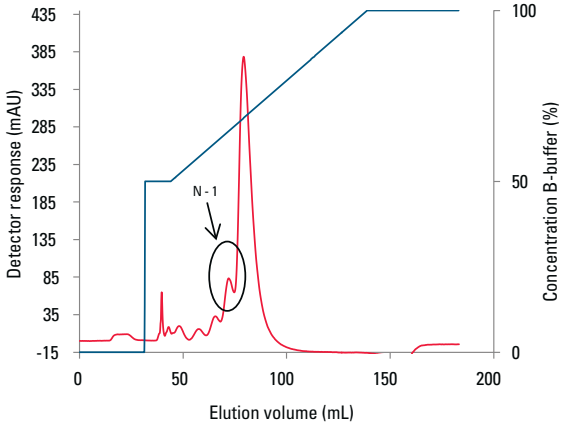
Table 10 shows the different particle sizes that are available in the TSKgel and TOYOPEARL anion exchange resins used for oligonucleotides, and the cation exchange resins used for peptide purifications. The relative binding capacities and predicted resolution of the different particle size resins are depicted by a series of "+" characters. The more "+" characters listed in the table the better one resin is relative to another for that parameter. If a process is developed using one of the resins and more resolution is needed, select an appropriate smaller particle size product. Similarly if more product throughput is needed and resolution is not a critical issue, a larger particle size resin can be selected.

The very high capacity TOYOPEARL GigaCap Q-650 resins (also shown in Table 10) can be used for oligonucleotide purifications, although the selectivity of this resin is somewhat different than the TSKgel and TOYOPEARL SuperQ-type resins. As seen in Figures 22-27, the TOYOPEARL GigaCap Q-650S performs similarly to the TSKgel SuperQ-5PW (20) resin for the purification of oligonucleotides. Table 11 compares the performance of these two resins for purity and recovery of an oligonucleotide from crude feedstock.

Table 10: Oligonucleotide purification products

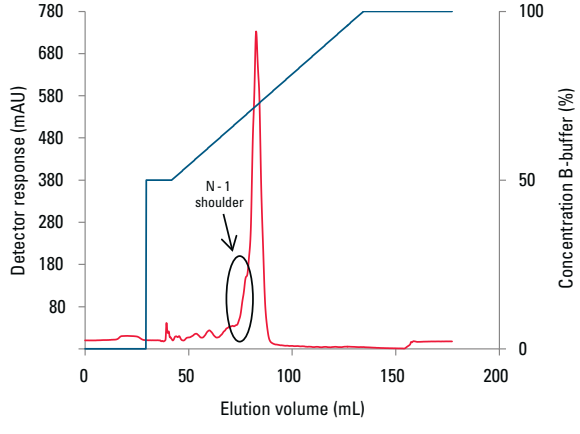
Resin	Bead size (mean µm)	Binding capacity g DNA oligo/L	Resolution	Bead type	Attachment method
TSKgel SuperQ-5PW (20)	20	45	+++++	methacrylic	Type A
TSKgel SuperQ-5PW (30)	30	40	++++	methacrylic	Type A
TOYOPEARL SuperQ-650S	35	54	+++	methacrylic	Type A
TOYOPEARL GigaCap Q-650S	35	40	+++	methacrylic	Type B
TOYOPEARL SuperQ-650M	65	50	++	methacrylic	Type A
TOYOPEARL GigaCap Q-650M	75	55	++	methacrylic	Type B
TOYOPEARL SuperQ-650C	100	50+ (est.)	+	methacrylic	Type A
TOYOPEARL Q-600 C AR	100	50	+	methacrylic	Type C

Figure 22: TSKgel SuperQ-5PW (20), 1.0 mg load



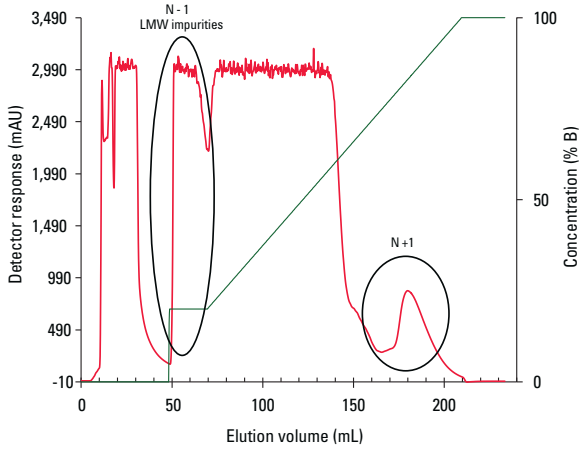
Resin: **TSKgel SuperQ-5PW (20)**
Column size: 6.6 mm ID × 18.5 cm (6.3 mL)
Mobile phase: Buffer A: 20 mmol/L NaOH
Buffer B: 20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient: 50 % B (2 CV)
50-100 % B (15 CV)
100 % B (2 CV)
Flow rate: 200 cm/h (1.14 mL/min)
Detection: UV @ 254 nm
Sample: crude phosphorothioate deoxyoligonucleotide
Sample load: 1.0 mg

Figure 23: TOYOPEARL GigaCap Q-650S, 1.0 mg load



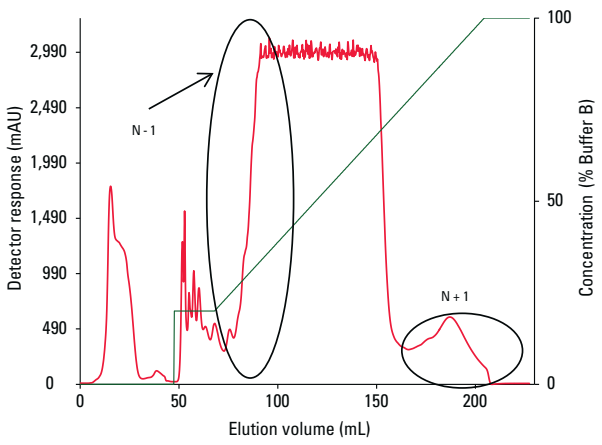
Resin: **TOYOPEARL GigaCap Q-650S**
Column size: 6.6 mm ID × 18.5 cm (6.3 mL)
Mobile phase: Buffer A: 20 mmol/L NaOH
Buffer B: 20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient: 50 % B (2 CV)
50-100 % B (15 CV)
100 % B (2 CV)
Flow rate: 200 cm/h (1.14 mL/min)
Detection: UV @ 254 nm
Sample: crude phosphorothioate deoxyoligonucleotide
Sample load: 1.0 mg

Figure 24: Purification of oligonucleotide at 80 % DBC on TSKgel SuperQ-5PW (20) resin



Resin:	**TSKgel SuperQ-5PW (20)**
Column size:	6.6 mm ID × 18.5 cm (6.3 mL)
Mobile phase:	Buffer A: 20 mmol/L NaOH
	Buffer B: 20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient:	20 % B (2 CV)
	20-100 % B (20 CV)
	100 % B (2 CV)
Flow rate:	200 cm/h (1.14 mL/min)
Detection:	UV @ 254 nm
Sample:	crude phosphorothioate deoxyoligonucleotide
Sample load:	235 mg

Figure 25: Purification of oligonucleotide at 80 % DBC on TOYOPEARL GigaCap Q-650S resin



Resin:	**TOYOPEARL GigaCap Q-650S**
Column size:	6.6 mm ID × 18 cm (6.16 mL)
Mobile phase:	Buffer A: 20 mmol/L NaOH
	Buffer B: buffer A + 3.0 mol/L NaCl
Gradient:	step to 20 % B (2 CV)
	20 % - 100 % B (20 CV)
	100 % B (2 CV)
Flow rate:	200 cm/h (1.14 mL/min)
Detection:	UV @ 254 nm
Sample:	crude phosphorothioate deoxyoligonucleotide
Sample load:	181.4 mg

Figure 26: TSKgel SuperQ-5PW (20) resin: 80 % DBC elution peak with fraction purity histogram

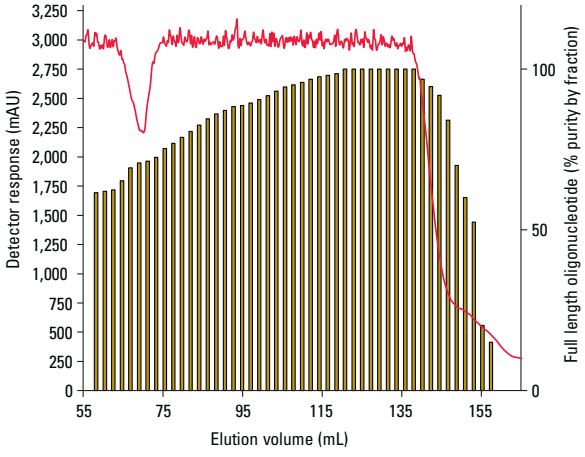


Figure 27: TOYOPEARL GigaCap Q-650S resin: 80 % DBC elution peak with fraction purity histogram

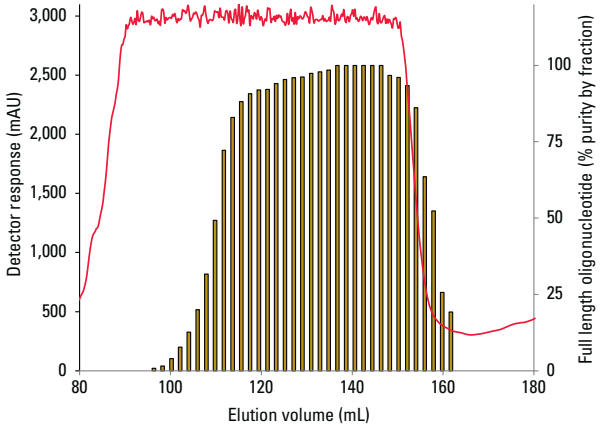


Table 11: Oligonucleotide purity and yield from 80% DBC purifications

Resin	Crude oligo purity	Final oligo purity	% Yield
TSKgel SuperQ-5PW (20)	66.5 %	96.4 %	72.5 %
TOYOPEARL GigaCap Q-650S	66.5 %	96.9 %	81.3 %

Peptide Purifications

Cation exchange chromatography is commonly used for peptide purification. Table 12 shows the same particle size profile availability of TOYOPEARL and TSKgel resins functionalized with the cation exchange SP ligand. Based on the needs for capacity and resolution, an appropriate SP resin should be selected for a particular peptide application.

Table 12: Peptide purification products

Resin	Bead size (mean µm)	Binding capacity	Resolution	Bead type	Attachment method
TSKgel SP-5PW (20)	20	++	+++++	methacrylic	Traditional
TSKgel SP-5PW (30)	30	++	++++	methacrylic	Traditional
TSKgel SP-3PW (30)	30	++	++++	methacrylic	Traditional
TOYOPEARL SP-650S	35	++++	+++	methacrylic	Traditional
TOYOPEARL SP-650M	65	++++	++	methacrylic	Traditional
TOYOPEARL SP-650C	100	++++	+	methacrylic	Traditional
TOYOPEARL GigaCap S-650S	35	+++++	+++	methacrylic	Type B
TOYOPEARL GigaCap S-650M	75	+++++	++	methacrylic	Type B

Insulin Purification

TSKgel SP-3PW (30) resin was developed as a higher resolving and higher capacity resin for insulin purification. Table 13 compares the capacity of this new resin to TSKgel SP-5PW (30) resin and SOURCE 30S resin. The improved resolving power of TSKgel SP-3PW (30) resin is demonstrated in Figure 28.

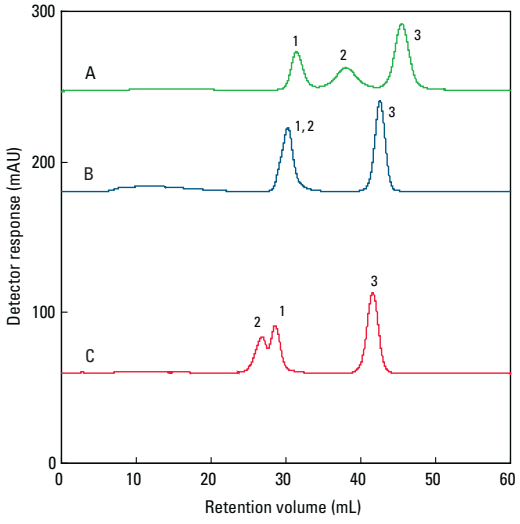
Table 13: Insulin dynamic binding capacity comparison

Resin	TSKgel SP-3PW (30)	TSKgel SP-5PW (30)	SOURCE 30S
Matrix	polymethacrylate	polymethacrylate	polystyrene divinylbenzene
Particle size	30 µm	30 µm	30 µm
Insulin capacity	49 g/L	24 g/L	45 g/L
Pore size	25 nm	100 nm	NR

Dynamic binding capacities were determined at 10 % breakthrough

Column size:	4.6 mm ID × 7.5 cm
Mobile phase:	gradient elution with 1-propanol by acidic buffer, pH 3.0 containing neutral salt
Flow rate:	270 cm/h (0.75 mL/min)
Sample:	recombinant insulin (7.2 g/L)

Figure 28: Selectivity comparison - insulin



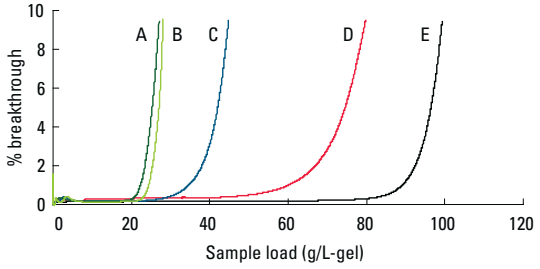
Resins: **A. TSKgel SP-3PW (30)**
B. SOURCE 30S
C. TSKgel SP-5PW (30)

Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	Buffer A: 0.02 mol/L sodium citrate buffer, pH 3.2 + ethanol = 8/2 (v/v)
	Buffer B: 0.02 mol/L sodium citrate buffer, pH 3.2 + 1.0 mol/L NaCl/ethanol = 8/2 (v/v)
Gradient:	60 min linear gradient from buffer A to buffer B
Flow rate:	136 cm/h (1.0mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	1. trypsinogen 2. insulin 3. lysozyme
Sample vol.:	100 µL (0.5 g/L each)

PEGylated Proteins

Ion exchange resins are frequently used for the purification of PEGylated proteins. Figure 29 shows the breakthrough curves of five TOYOPEARL cation exchange resins for mono-PEGylated lysozyme. The selectivities of TOYOPEARL GigaCap CM-650M and TOYOPEARL GigaCap S-650M resins for native lysozyme and its mono-PEGylated counterpart are shown in Figure 30.

Figure 29: Breakthrough curves of mono-PEGylated lysozyme using TOYOPEARL cation exchange resins

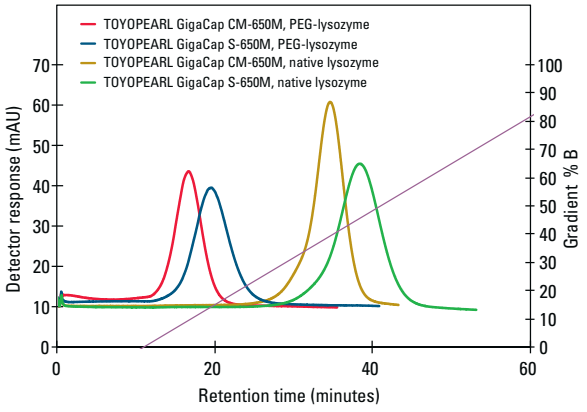


Resins: **A. TOYOPEARL SP-650M**
B. TOYOPEARL CM-650M
C. TOYOPEARL SP-550C
D. TOYOPEARL GigaCap CM-650M
E. TOYOPEARL GigaCap S-650M

Column size: 6 mm ID × 40 mm
Mobile phase: Buffer A: 20 mmol/L phosphate buffer, pH 7.0
Buffer B: 20 mmol/L phosphate buffer, pH 7.0 + 0.5 mol/L NaCl
Flow rate: 212 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Sample: mono-PEGylated lysozyme, 1.0 g/L (PEG MW= 5 kDa)

Dynamic binding capacities were determined at 10 % breakthrough

Figure 30: Selectivity comparison between native protein and mono-PEGylated protein on TOYOPEARL GigaCap resins



Resins: **TOYOPEARL GigaCap S-650M**
TOYOPEARL GigaCap CM-650M

Column size: 6 mm ID × 4 cm
Mobile phase: Buffer A: 50 mmol/L phosphate buffer, pH 7.0
Buffer B: 50 mmol/L phosphate buffer, pH 7.0 + 0.5 mol/L NaCl

Gradients:

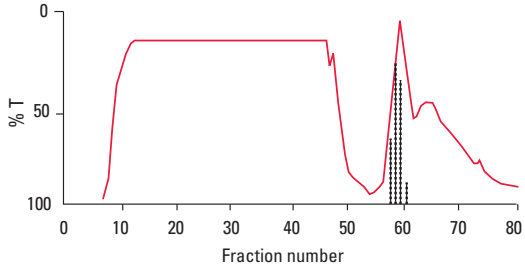
TOYOPEARL GigaCap S-650M	TOYOPEARL GigaCap CM-650M
10 minute 100 % buffer A	10 minute 100 % buffer A
60 minutes 0 %B to 100 %B	60 minutes 0 %B to 50 %B

Flow rate: 212 cm/h (1 mL/min)
Samples: native lysozyme, 5 g/L
mono-PEGylated lysozyme, 5 g/L (PEG MW= 5 kDa)

Antibody Purification

Klapper *et al.* reported the use of the TOYOPEARL CM-650S for the purification of monoclonal antibodies.[1] Figure 31 shows the elution profile of monoclonal antibody supernatant. Antibody activity is represented in the figure by the black bars.

Figure 31: Separation of monoclonal antibody cell culture supernatant



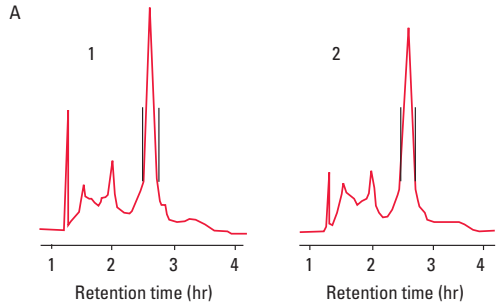
Resin:	**TOYOPEARL CM-650S**
Column size:	16 mm ID × 6 cm
Mobile phase:	Buffer A: 20 mmol/L sodium acetate buffer, pH 5.5 Buffer B: 20 mmol/L sodium acetate buffer, pH 5.5 + 0.5 mol/L NaCl
Gradient:	linear gradient from buffer A to buffer B in 200 mL total volume
Flow rate:	173 cm/h (5.8 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	100 mL of monoclonal antibody cell culture supernatant

[1]Klapper, D.; Osgood, S.; Esch, R.; Olson, J. Use of new HPLC resins to solve old problems. J. of Liquid Chromatography. 1986, 9, (8), 1613-1633.

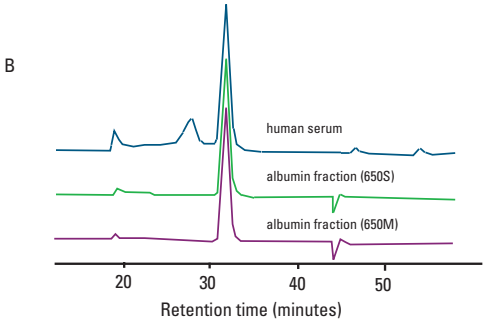
Blood Proteins

The separations of human serum on both TOYOPEARL DEAE-650M and TOYOPEARL DEAE-650S are shown in Figure 32. The albumin fractions were collected (between the two vertical lines) and were analyzed via size exclusion chromatography on two TSKgel G3000SW columns in series. As seen in the figure, the albumin fractions contain small amounts of a high formula weight contaminant which is probably α-globulin.[2] Analytical IEX (not shown) demonstrated that the albumin peaks were fairly homogeneous.

Figure 32: Separation of human serum and albumin fractions



Resins:	**1. TOYOPEARL DEAE-650S** **2. TOYOPEARL DEAE-650M**
Column size:	16 mm ID × 15 cm
Mobile phase:	Buffer A: 50 mmol/LTris-HCl buffer, pH 8.6 Buffer B: 50 mmol/L Tris-HCl buffer, pH 8.6 + 0.5 mol/L NaCl
Gradient:	linear gradient from buffer A to buffer B in 200 mL total volume
Flow rate:	45 cm/h (1.5 mL/min)
Detection:	UV @ 280 nm
Temperature:	25 ˚C
Sample:	human serum



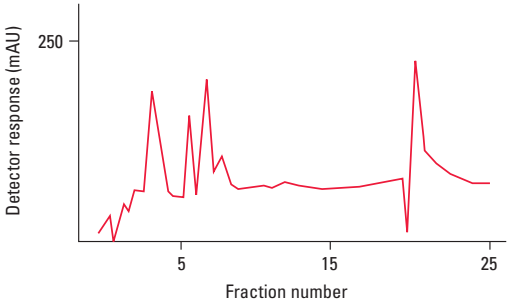
Column:	**TSKgel G3000SW, 7.5 mm ID × 30 cm × 2 in series**
Mobile phase:	0.1 mol/L phosphate buffer, pH 6.8 + 0.1 mol/L sodium sulfate
Detection:	UV @ 280 nm
Temperature:	25 ˚C
Sample:	1. crude human serum 2. albumin fraction from TOYOPEARL DEAE-650S 3. albumin fraction from TOYOPEARL DEAE-650M

[2]Kato, Y.; Nakamura, K.; Hashimoto, T. Characterization of TSK-GEL DEAE-Toyopearl 650 Ion Exchanger. J. Chromatogr. 1982, 245, 193-211.

Tryptic Digests

Tryptic fragments from radiolabeled human immunoglobulin light chain can be separated using anion exchange chromatography on TOYOPEARL DEAE-650S.[1] Figure 33 shows the elution profile of a tryptic digest fraction from an SEC column run on TOYOPEARL DEAE-650S. The recovery of the radiolabeled product was greater than 90 %.

Figure 33: Separation of tryptic digest peptide mixture



Resin:	**TOYOPEARL DEAE-650S**
Column size:	6 mm ID × 12 cm
Mobile phase:	pyridine/N-ethyl morpholine
Flow rate:	212 cm/h (1 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	enzymatic digest of immunoglobulin L chain

[1]Klapper, D.; Osgood, S.; Esch, R.; Olson, J. Use of new HPLC resins to solve old problems. J. of Liquid Chromatography. 1986, 9, (8), 1613-1633.

A selection of screening tools are available for TOYOPEARL and TSKgel IEX resins. See the Process Development Products section of this Product Guide for details.

Ordering Information

Anion exchange resins:

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical lysozyme capacity (g/L)
TOYOPEARL and TOYOPEARL GigaCap Anion Exchange Resins					
0043271	TOYOPEARL QAE-550C	100	50 - 150	0.28 - 0.38	60 - 80
0014026	TOYOPEARL QAE-550C	250			
0014704	TOYOPEARL QAE-550C	1,000			
0014027	TOYOPEARL QAE-550C	5,000			
0018365	TOYOPEARL QAE-550C	50,000			
0021985	TOYOPEARL Q-600C AR	100	50 - 150	0.15 - 0.20	≥ 120
0021986	TOYOPEARL Q-600C AR	250			
0021987	TOYOPEARL Q-600C AR	1,000			
0021988	TOYOPEARL Q-600C AR	5,000			
0021989	TOYOPEARL Q-600C AR	50,000			
0021854	TOYOPEARL GigaCap Q-650M	100	50 - 100	0.20 - 0.30	≥ 162
0021855	TOYOPEARL GigaCap Q-650M	250			
0021856	TOYOPEARL GigaCap Q-650M	1,000			
0021857	TOYOPEARL GigaCap Q-650M	5,000			
0021858	TOYOPEARL GigaCap Q-650M	50,000			
0019823	TOYOPEARL SuperQ-650S	25	20 - 50	0.20 - 0.30	105 - 155
0017223	TOYOPEARL SuperQ-650S	250			
0017224	TOYOPEARL SuperQ-650S	1,000			
0017225	TOYOPEARL SuperQ-650S	5,000			
0019679	TOYOPEARL SuperQ-650S	50,000			
0043205	TOYOPEARL SuperQ-650M	100	40 - 90	0.20 - 0.30	105 - 155
0017227	TOYOPEARL SuperQ-650M	250			
0017228	TOYOPEARL SuperQ-650M	1,000			
0017229	TOYOPEARL SuperQ-650M	5,000			
0021311	TOYOPEARL SuperQ-650M	50,000			
0043275	TOYOPEARL SuperQ-650C	100	50 - 150	0.20 - 0.30	105 - 155
0017231	TOYOPEARL SuperQ-650C	250			
0017232	TOYOPEARL SuperQ-650C	1,000			
0017233	TOYOPEARL SuperQ-650C	5,000			
0019804	TOYOPEARL DEAE-650S	25	20 - 50	0.08 - 0.12	25 - 35
0007472	TOYOPEARL DEAE-650S	250			
0014692	TOYOPEARL DEAE-650S	1,000			
0007973	TOYOPEARL DEAE-650S	5,000			
0021483	TOYOPEARL DEAE-650S	50,000			

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical lysozyme capacity (g/L)
0043201	TOYOPEARL DEAE-650M	100	40 - 90	0.08 - 0.12	25 - 35
0007473	TOYOPEARL DEAE-650M	250			
0014693	TOYOPEARL DEAE-650M	1,000			
0007974	TOYOPEARL DEAE-650M	5,000			
0018367	TOYOPEARL DEAE-650M	50,000			
0007988	TOYOPEARL DEAE-650C	250	50 - 150	0.05 - 0.11	25 - 35
0014694	TOYOPEARL DEAE-650C	1,000			
0007989	TOYOPEARL DEAE-650C	5,000			
0022853	TOYOPEARL DEAE-650C	50,000			
0022865	TOYOPEARL GigaCap DEAE-650M	100	50 - 100	0.15 - 0.25	≥ 156
0022866	TOYOPEARL GigaCap DEAE-650M	250			
0022867	TOYOPEARL GigaCap DEAE-650M	1,000			
0022868	TOYOPEARL GigaCap DEAE-650M	5,000			
0022869	TOYOPEARL GigaCap DEAE-650M	50,000			
0022881	TOYOPEARL GigaCap Q-650S	25	20 - 50	0.14 - 0.24	≥162
0022882	TOYOPEARL GigaCap Q-650S	250			
0022883	TOYOPEARL GigaCap Q-650S	1,000			
0022884	TOYOPEARL GigaCap Q-650S	5,000			
0022885	TOYOPEARL GigaCap Q-650S	50,000			
TSKgel Anion Exchange Resins					
0043383	TSKgel SuperQ-5PW (20)	25	15 - 25	0.12 - 0.18	52 - 88
0018535	TSKgel SuperQ-5PW (20)	250			
0018546	TSKgel SuperQ-5PW (20)	1,000			
0018547	TSKgel SuperQ-5PW (20)	5,000			
0021919	TSKgel SuperQ-5PW (20)	25,000			
0021920	TSKgel SuperQ-5PW (20)	50,000			
0043283	TSKgel SuperQ-5PW (30)	25	20 - 40	0.12 - 0.18	52 - 88
0018536	TSKgel SuperQ-5PW (30)	250			
0018548	TSKgel SuperQ-5PW (30)	1,000			
0018549	TSKgel SuperQ-5PW (30)	5,000			
0043381	TSKgel DEAE-5PW (20)	25	15 - 25	0.05 - 0.11	25 - 45
0014710	TSKgel DEAE-5PW (20)	250			
0014711	TSKgel DEAE-5PW (20)	1,000			
0018436	TSKgel DEAE-5PW (20)	5,000			
0043281	TSKgel DEAE-5PW (30)	25	20 - 40	0.05 - 0.11	20 - 40
0014712	TSKgel DEAE-5PW (30)	250			
0014713	TSKgel DEAE-5PW (30)	1,000			
0018370	TSKgel DEAE-5PW (30)	5,000			

Cation exchange resins:

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical lysozyme capacity (g/L)
TOYOPEARL and TOYOPEARL GigaCap Cation Exchange Resins					
0021833	TOYOPEARL GigaCap S-650M	100	50 - 100	0.14 - 0.18	136 - 176 human γ-globulin
0021834	TOYOPEARL GigaCap S-650M	250			
0021835	TOYOPEARL GigaCap S-650M	1,000			
0021836	TOYOPEARL GigaCap S-650M	5,000			
0021837	TOYOPEARL GigaCap S-650M	50,000			
0022875	TOYOPEARL GigaCap S-650S	25	20 - 50	0.15 - 0.25	≥ 150 human IgG
0022876	TOYOPEARL GigaCap S-650S	250			
0022877	TOYOPEARL GigaCap S-650S	1,000			
0022878	TOYOPEARL GigaCap S-650S	5,000			
0022879	TOYOPEARL GigaCap S-650S	50,000			
0021946	TOYOPEARL GigaCap CM-650M	100	50 - 100	0.18 - 0.28	≥ 110 γ-globulin
0021947	TOYOPEARL GigaCap CM-650M	250			
0021948	TOYOPEARL GigaCap CM-650M	1,000			
0021949	TOYOPEARL GigaCap CM-650M	5,000			
0021950	TOYOPEARL GigaCap CM-650M	50,000			
0043272	TOYOPEARL SP-550C	100	50 - 150	0.14 - 0.18	80 - 120
0014028	TOYOPEARL SP-550C	250			
0014705	TOYOPEARL SP-550C	1,000			
0014029	TOYOPEARL SP-550C	5,000			
0018366	TOYOPEARL SP-550C	50,000			
0019822	TOYOPEARL SP-650S	25	20 - 50	0.13 - 0.17	40 - 60
0008437	TOYOPEARL SP-650S	250			
0014698	TOYOPEARL SP-650S	1,000			
0008438	TOYOPEARL SP-650S	5,000			
0021477	TOYOPEARL SP-650S	50,000			
0043202	TOYOPEARL SP-650M	100	40 - 90	0.13 - 0.17	40 - 60
0007997	TOYOPEARL SP-650M	250			
0014699	TOYOPEARL SP-650M	1,000			
0007998	TOYOPEARL SP-650M	5,000			
0018369	TOYOPEARL SP-650M	50,000			
0007994	TOYOPEARL SP-650C	250	50 - 150	0.12 - 0.18	35 - 55
0014700	TOYOPEARL SP-650C	1,000			
0007995	TOYOPEARL SP-650C	5,000			
0019803	TOYOPEARL CM-650S	25	20 - 50	0.08 - 0.12	30 - 50
0007474	TOYOPEARL CM-650S	250			

Part #	Product description	Container size (mL)	Bead diameter (µm)	Ion Exchange Capacity (eq/L)	Typical lysozyme capacity (g/L)
0014695	TOYOPEARL CM-650S	1,000	20 - 50	0.08 - 0.12	30 - 50
0007971	TOYOPEARL CM-650S	5,000			
0043203	TOYOPEARL CM-650M	100	40 - 90	0.08 - 0.12	30 - 50
0007475	TOYOPEARL CM-650M	250			
0014696	TOYOPEARL CM-650M	1,000			
0007972	TOYOPEARL CM-650M	5,000			
0019839	TOYOPEARL CM-650M	50,000			
0007991	TOYOPEARL CM-650C	250	50 - 150	0.05 - 0.11	25 - 45
0014697	TOYOPEARL CM-650C	1,000			
0007992	TOYOPEARL CM-650C	5,000			
0019329	TOYOPEARL CM-650C	50,000			
0021804	TOYOPEARL MegaCap II SP-550EC	100	100 - 300	0.14 - 0.18	60 - 90*
0021805	TOYOPEARL MegaCap II SP-550EC	250			
0021806	TOYOPEARL MegaCap II SP-550EC	1,000			
0021807	TOYOPEARL MegaCap II SP-550EC	5,000			
0021808	TOYOPEARL MegaCap II SP-550EC	50,000			
TSKgel Cation Exchange Resins					
0043382	TSKgel SP-5PW (20)	25	15 - 25	0.06 - 0.12	20 - 40
0014714	TSKgel SP-5PW (20)	250			
0014715	TSKgel SP-5PW (20)	1,000			
0018435	TSKgel SP-5PW (20)	5,000			
0043282	TSKgel SP-5PW (30)	25	20 - 40	0.06 - 0.12	20 - 40
0014716	TSKgel SP-5PW (30)	250			
0014717	TSKgel SP-5PW (30)	1,000			
0018384	TSKgel SP-5PW (30)	5,000			
0021807	TSKgel SP-5PW (30)	50,000			
0021976	TSKgel SP-3PW (30)	25	20 - 40	0.07 - 0.12	≥ 65 insulin
0021977	TSKgel SP-3PW (30)	250			
0021978	TSKgel SP-3PW (30)	1,000			
0021979	TSKgel SP-3PW (30)	5,000			
0021980	TSKgel SP-3PW (30)	50,000			

* Adsorption capacity for insulin: 90-120 g/L resin

TOYOPEARL Butyl-600M		
TOYOPEARL Butyl-650C	TOYOPEARL Butyl-650M	TOYOPEARL Butyl-650S
TOYOPEARL Ether-650M	TOYOPEARL Ether-650S	
TSKgel Ether-5PW (20)	TSKgel Ether-5PW (30)	
TOYOPEARL Hexyl-650C		
TOYOPEARL Phenyl-600M		
TOYOPEARL Phenyl-650C	TOYOPEARL Phenyl-650M	TOYOPEARL Phenyl-650S
TSKgel Phenyl-5PW (20)	TSKgel Phenyl-5PW (30)	
TOYOPEARL PPG-600M		
TOYOPEARL SuperButyl-550C		

The role of Hydrophobic Interaction Chromatography in Process Purification

Hydrophobic interaction chromatography (HIC) is a powerful tool for the process purification of biomolecules. The technique utilizes the accessible hydrophobic regions located on protein surfaces and their interactions with a weakly hydrophobic stationary phase. HIC is an excellent complement to ion exchange and size exclusion chromatography particularly when protein isoforms exist or when feedstock impurities are of similar isoelectric point or molar mass. The selectivity differences exploited by HIC can also be used after affinity separations in which closely related proteins with similar recognition sites are not distinguishable by the affinity ligand.

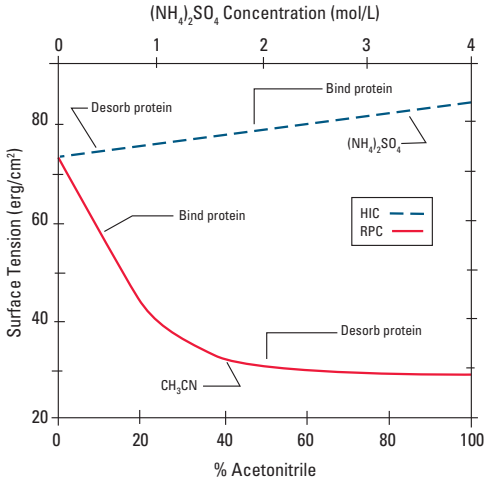
Proteins and other molecules with hydrophobic surfaces are attracted to the hydrophobic ligands of HIC resins. Proteins are bound to the resin by employing an aqueous high salt mobile phase. The salt conditions contribute to a lyotropic effect which allows the proteins to bind to the lower surface coverage of a hydrophobic ligand. Proteins are eluted by the simple technique of decreasing the salt concentration. Most therapeutic targets are eluted in a low salt or a no salt buffer.

During elution, the energy of interaction for a HIC step is less than that of a reversed phase chromatography (RPC) step. One means of gauging the relative binding energy between the two techniques is to measure the surface tension of the two sets of binding and elution conditions. Figure 1 provides a comparison of the surface tension generated by HIC and RPC elution systems.[1] Since HIC separates under milder eluting conditions, biological activity is typically retained.

TOYOPEARL Hydrophobic Interaction Chromatography Resins

TOYOPEARL HIC resins are functionalized versions of the TOYOPEARL HW size exclusion resins and are therefore based on hydroxylated polymethacrylic polymer beads. Tosoh Bioscience offers five HIC ligands featuring different degrees of hydrophobicity and selectivity. Table 1 lists the properties of these TOYOPEARL HIC resins. The hydrophobicity of TOYOPEARL HIC resins increases through the ligand series: ether, PPG (polypropylene glycol), phenyl, butyl, and hexyl (Figure 2).

Figure 1: The surface tension of aqueous solutions used in HIC and RPC



Mode	Gradient (Typical)	Δ Surface Tension (erg/cm²)
HIC	1.8 to 0 mol/L $(NH_4)_2SO_4$/ aqueous buffer	4
RPC	10 to 50% ACN/ 0.1%TFA	23

[1]C. Horvath et. al., Separation Processes in Biotechnology, Volume 9; Asenjo, J. ed.; Marcel Dekker, Inc.: New York, 1990, p 447.

Figure 2: Available HIC ligands

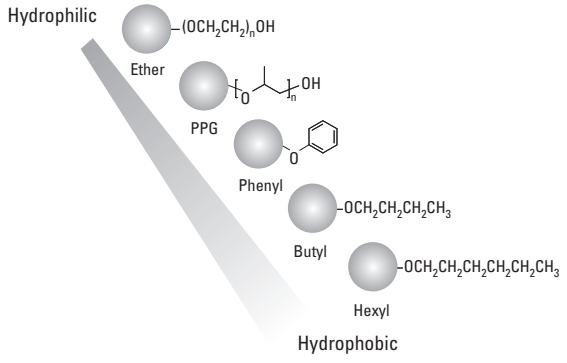
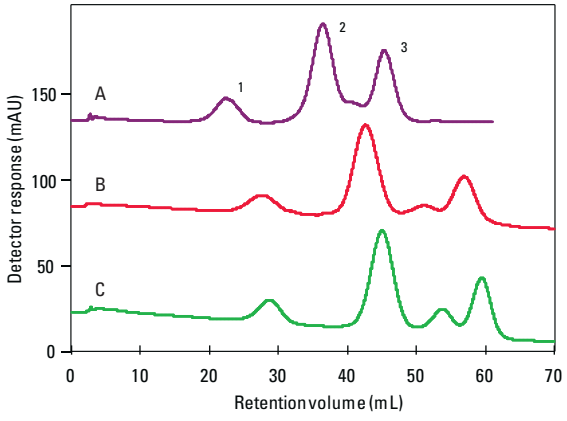


Table 1: Properties of TOYOPEARL HIC resins

TOYOPEARL resin	Hydrophobicity	Base bead	Pore size (nm)	Bead diameter (µm)	Ligand type	DBC (g/L)	Pressure rating
Ether-650S	+	HW-65	100	20 - 50	Ether	10-30	0.3 MPa
Ether-650M	+	HW-65	100	40 - 90	Ether	10-30	0.3 MPa
PPG-600M	++	HW-60	75	40 - 90	Polypropylene glycol	45 - 55	0.3 MPa
Phenyl-600M	+++	HW-60	75	40 - 90	Phenyl	45 - 65	0.3 MPa
Phenyl-650S	+++	HW-65	100	20 - 50	Phenyl	30 - 50	0.3 MPa
Phenyl-650M	+++	HW-65	100	40 - 90	Phenyl	30 - 50	0.3 MPa
Phenyl-650C	+++	HW-65	100	50 - 150	Phenyl	30 - 50	0.3 MPa
Butyl-650S	++++	HW-65	100	20 - 50	Butyl	30 - 50	0.3 MPa
Butyl-650M	++++	HW-65	100	40 - 90	Butyl	30 - 50	0.3 MPa
Butyl-650C	++++	HW-65	100	50 - 150	Butyl	30 - 50	0.3 MPa
Butyl-600M	++++	HW-60	75	40 - 90	Butyl	40 - 60	0.3 MPa
SuperButyl-550C	++++	HW-55	50	50 - 150	Butyl	52 - 70	0.3 MPa
Hexyl-650C	+++++	HW-65	100	50 - 150	Hexyl	30 - 50	0.3 MPa

Three HIC ligands are available in the TOYOPEARL -600 resin format: PPG, phenyl, and butyl. The selectivities of TOYOPEARL Butyl-600M, TOYOPEARL PPG-600M and the TOYOPEARL Phenyl-600M resins are shown in Figure 3. Available in the TOYOPEARL -650 series are the following four HIC ligands: hexyl, butyl, phenyl, and ether. The remaining ligand available in the TOYOPEARL HIC resin line is SuperButyl-550.

Figure 3: Comparison of TOYOPEARL -600M resins

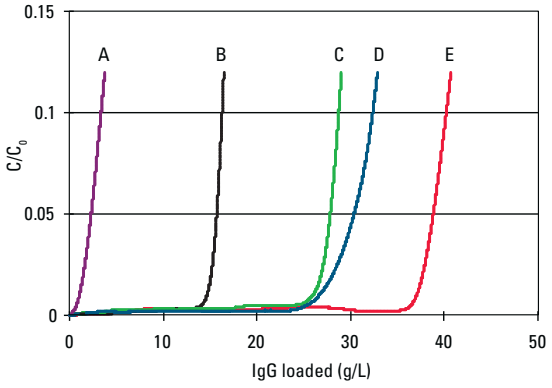


Resins: **A. TOYOPEARL PPG-600M**
B. TOYOPEARL Phenyl-600M
C. TOYOPEARL Butyl-600M

Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 1.8 mol/L $(NH_4)_2SO_4$ + 0.1 mol/L sodium phosphate buffer, pH 7.0
Buffer B: 0.1 mol/L sodium phosphate buffer, pH 7.0
Gradient: 60 min linear gradient from buffer A to B
Flow rate: 136 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Temperature: ambient
Samples: 1 g/L of: 1. ribonuclease A 2.lysozyme 3. α-chymotrypsinogen
Load volume: 100 µL

A comparison of the dynamic binding capacities (DBCs) of the TOYOPEARL -600 resins with TOYOPEARL Phenyl-650M is shown in Figure 4. Figure 5 compares the selectivities of the TOYOPEARL Phenyl-600M and TOYOPEARL Phenyl-650M resins with an agarose based phenyl resin. The narrower pore diameter of TOYOPEARL SuperButyl-550C resin (based on the 50 nm pore diameter TOYOPEARL HW-55 resin) is recommended for the analysis of smaller molecules such as lysozyme (1.2×10^4 Da). A comparison of the DBC of TOYOPEARL SuperButyl-550C resin with other TOYOPEARL HIC resins is shown in Figures 6 and 7.

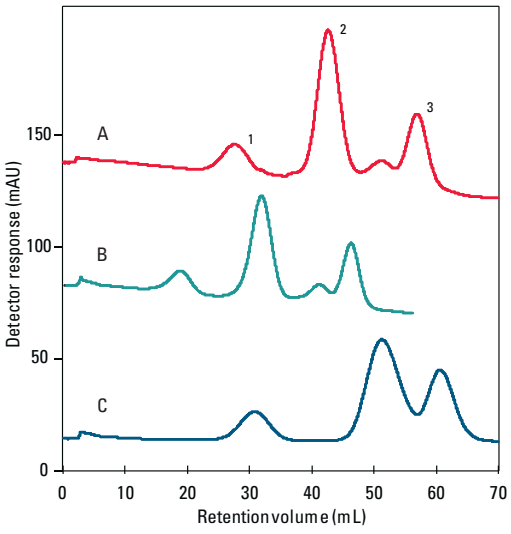
Figure 4: Breakthrough curves of polyclonal IgG on various HIC resins



Resins: **A. TOYOPEARL PPG-600M**
B. TOYOPEARL Phenyl-600M
C. TOYOPEARL Butyl-600M
D. Competitor Phenyl Agarose
E. TOYOPEARL Phenyl-600M
Column size: 7.8 mm ID × 20 cm
Mobile phase: 0.1 mol/L sodium phosphate buffer, pH 7.0 + 0.8 mol/L $(NH_4)_2SO_4$
Flow rate: 300 cm/h (2.4 mL/min)
Detection: UV @ 280 nm
Temperature: 25 °C
Samples: 1.0 g/L polyclonal IgG

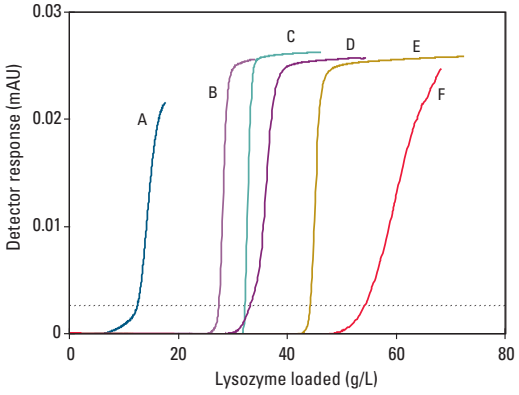
DBC was calculated at 10 % breakthrough

Figure 5: Selectivity comparison of phenyl-type resins



Resins: **A. TOYOPEARL Phenyl-600M**
B. TOYOPEARL Phenyl-650M
C. Competitor Phenyl Agarose
Column size: 7.5 mm ID × 7.5 cm
Mobile phase: Buffer A: 1.8 mol/L $(NH_4)_2SO_4$ + 0.1 mol/L sodium phosphate buffer, pH 7.0
Buffer B: 0.1 mol/L sodium phosphate buffer, pH 7.0
Gradient: 60 min linear gradient from buffer A to B
Flow rate: 136 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Temperature: ambient
Sample: 1.0 g/L of: 1. ribonuclease A 2.lysozyme 3. α-chymotrypsinogen
Load volume: 100 µL

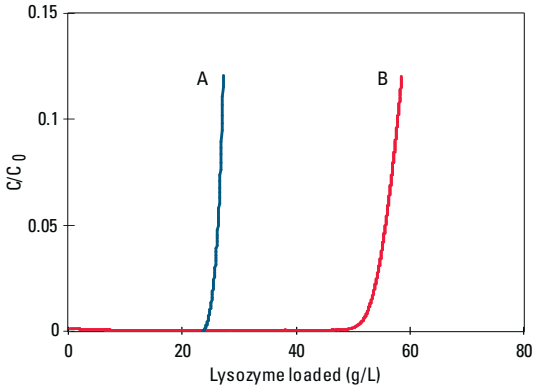
Figure 6: Typical dynamic binding capacities for lysozyme



Resins:	binding capacity (g/L) (10 % breakthrough)
A. TOYOPEARL Ether-650M	12.5
B. TOYOPEARL Phenyl-650M	27.5
C. TOYOPEARL Butyl-650M	32.2
D. TOYOPEARL Hexyl-650C	33.2
E. TOYOPEARL PPG-600M	44.2
F. TOYOPEARL SuperButyl-550C	54.3

Column size: 7.8 mm ID × 20 cm
Mobile phase: 1.8 mol/L sodium sulfate + 0.1 mol/L phosphate buffer, pH 7.0
Flow rate: 100 cm/h (0.8 mL/min)
Detection: UV @ 280 nm
Temperature: ambient
Sample: 1 g/L lysozyme
Sample load: as indicated in figure

Figure 7: TOYOPEARL Phenyl-600M breakthrough curve (lysozyme)



Resins:	binding capacity (g/L) (10 % breakthrough)
A. TOYOPEARL Phenyl-600M	58
B. TOYOPEARL Phenyl-650M	27

Column size: 7.8 mm ID × 20 cm
Mobile phase: 1.8 mol/L $(NH_4)_2SO_4$ + 0.1 mol/L phosphate buffer, pH 7.0
Flow rate: 300 cm/h (2.4 mL/min)
Detection: UV @ 280 nm
Temperature: ambient
Sample: 1 g/L lysozyme
Sample load: as indicated in figure

The larger pore TOYOPEARL products such as TOYOPEARL Butyl-650 and TOYOPEARL Phenyl-650 resins are very useful for protein aggregate separation and removal. In addition, Tosoh Bioscience HIC resins are very effective in separating misfolded proteins from the native protein form. Because misfolded proteins will generally be more hydrophobic than the native protein, TOYOPEARL Butyl-650M resin is used frequently for the removal of misfolded proteins. In many cases, flow-through chromatography can be accomplished under eluent conditions binding the misfolded protein while allowing the native target protein to flow through the column.

Hydrophobic interaction is a very useful technique for the purification of monoclonal antibodies (mAbs), with their diverse hydrophobic nature. The range of HIC ligands of varying hydrophobicity available from Tosoh Bioscience (Figure 2) gives chromatographic developers a range of options for finding the right ligand for their target molecule.

TSKgel Hydrophobic Interaction Chromatography Resins

The same ether and phenyl ligands that are used for the TOYOPEARL resins are also available within the TSKgel HIC resin product line. Properties of TSKgel HIC resins are listed in Table 2. The TSKgel HIC resins use the same methacrylic polymer chemistry as the TOYOPEARL resins (Table 3) but have a higher degree of crosslinking, making for a more rigid bead. This is necessitated by the higher pressures generated when using smaller particles for chromatography. Greater crosslinking decreases the number of sites available for ligand attachment and thus a TSKgel resin will have a lower dynamic binding capacity than the corresponding TOYOPEARL resin. The polymeric structure of these products also makes them resistant to a wide range of pH conditions and mobile phase ionic strengths. In addition, the hydroxylated surface of the base bead reduces non-specific binding of proteins.

Table 3: Methacrylic base beads available for HIC

Pore size (nm)	5	12.5	40-50	75	100	>100	>170
Resin							
TOYOPEARL HW-type:	40	50	55	60	65	75	80
TSKgel PW-type:	G1000	G2000	G4000		G5000	G6000	

Increasing pore surface area

TOYOPEARL HIC resins are chemically stable from pH 1-13. This allows a constant packing volume over a wide range of salt concentrations and cleaning in place (CIP) with acid or base. Also, these resins can be run at elevated temperatures (4-60 °C) and are autoclavable at 121 °C.

Table 2: Properties of TSKgel HIC resins

TSKgel resin	Hydrophobicity	Base bead	Pore size (nm)	Bead diameter (µm)	Ligand type	DBC (g/L)	Pressure rating
Ether-5PW (20)	+	G5000PW	100	15 - 25	Ether	10 - 30	2.0 MPa
Ether-5PW (30)	+	G5000PW	100	20 - 40	Ether	10 - 30	2.0 MPa
Phenyl-5PW (20)	++	G5000PW	100	15 - 25	Phenyl	10 - 30	2.0 MPa
Phenyl-5PW (30)	++	G5000PW	100	20 - 40	Phenyl	10 - 30	2.0 MPa

Because TOYOPEARL and TSKgel HIC resins have the same backbone polymer chemistry, the selectivity for proteins and impurities will be unchanged. Table 4 shows the ligands and particle sizes available for TOYOPEARL and TSKgel HIC resins and is arranged in increasing levels of resolution by bead size (i.e. low, medium and high resolution). The semi-rigid polymeric backbone of TOYOPEARL and TSKgel HIC resins permits high flow rates for maximum throughput and productivity. TOYOPEARL HIC resins may be operated at pressures up to 0.3 MPa and TSKgel -5PW HIC resins may be operated up to 2.0 MPa. The pressure-flow characteristics for each particle size grade of TOYOPEARL Phenyl-650 resins are shown in Figure 8.

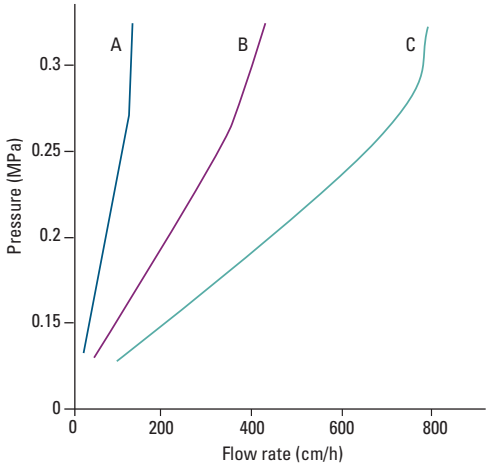
Resolution increases with decreasing particle size. Resin particle size is proportional to HETP and inversely proportional to the column efficiency and resolution of two peaks. TOYOPEARL HIC resins are available in three particle sizes, though not all ligands are available in each grade:

- S-grade = 35 µm (Superfine)
- M-grade = 65 µm (Fine)
- C-grade = 100 µm (Coarse)

Some processes, such as the purification of antibody-drug conjugates, require resins that are capable of higher resolution separations. For these separations, smaller diameter TOYOPEARL S-grade or TSKgel resins are preferred. TSKgel HIC resins are currently available in two ligands and two bead sizes:

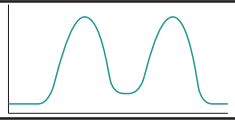
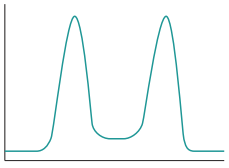
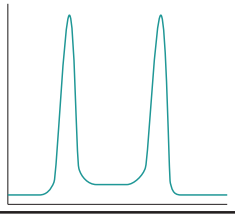
- TSKgel Ether-5PW (30) = 30 µm
- TSKgel Ether-5PW (20) = 20 µm
- TSKgel Phenyl-5PW (30) = 30 µm
- TSKgel Phenyl-5PW (20) = 20 µm

Figure 8: Pressure-flow curve for TOYOPEARL Phenyl-650 resins of various particle sizes



Resins: **A. TOYOPEARL Phenyl-650S**
B. TOYOPEARL Phenyl-650M
C. TOYOPEARL Phenyl-650C
Column size: 25 mm ID × 25 cm
Mobile phase: 2.0 mol/L $(NH_4)_2SO_4$
Flow rate: as indicated in figure

Table 4: Resolution of TOYOPEARL and TSKgel HIC resins

Resolution		Bead diameter (µm)	Pore size (nm)	HIC resin
Low		100	50 100 100 100	TOYOPEARL SuperButyl-550C TOYOPEARL Hexyl-650C TOYOPEARL Butyl-650C TOYOPEARL Phenyl-650C
Medium		65	75 75 75	TOYOPEARL Butyl-600M TOYOPEARL Phenyl-600M TOYOPEARL PPG-600M
		65	100 100 100	TOYOPEARL Butyl-650M TOYOPEARL Phenyl-650M TOYOPEARL Ether-650M
High		35	100 100 100	TOYOPEARL Butyl-650S TOYOPEARL Phenyl-650S TOYOPEARL Ether-650S
		30	100 100	TSKgel Phenyl-5PW (30) TSKgel Ether-5PW (30)
		20	100 100	TSKgel Phenyl-5PW (20) TSKgel Ether-5PW (20)

Parameters to Consider when Using Tosoh Bioscience HIC Resins

Coordinating the hydrophobicity of the therapeutic target to the resin hydrophobicity is critical for the best overall purification performance. Too hydrophobic a resin for a given protein can result in its irreversible binding to the resin or a loss of biological activity. Tables 5 and 6 show typical mass recovery and biological activity recovery data for TOYOPEARL HIC resins.

Table 5: High mass recovery (%) of proteins

Protein	TOYOPEARL resin		
	Ether-650M	Phenyl-650M	Butyl-650M
bovine serum albumin	84	62	76*
α-chymotrypsinogen	96	88*	90
cytochrome c	—	81*	87*
IgG	91	—	—
α-lactalbumin	90	—	—
lysozyme	94	92	85
ovalbumin	83	88	73
ribonuclease A	—	72*	82*

Procedure: A 200 mL sample containing 200 mg of protein was loaded onto a 7.5 mm ID × 7.5 cm column and eluted with a 60 minute gradient of 1.8 mol/L (*1.5 mol/L) to 0.0 mol/L ammonium sulfate in 0.1 mol/L sodium phosphate, pH 7.0. The mass recovery was determined spectrophotometrically at UV 280 nm and 25 °C.

Table 6: Recovery of enzymatic activity of proteins

TOYOPEARL resin	Protein	% Activity recovery
Phenyl-650	phytochrome	79
Butyl-650	halophilic protease	85
Butyl-650	poly (3-hydroxybutyrate) depolymerase	88
Butyl-650	aculeacin-A acylase	82
Butyl-650	opine dehydrogenase	81

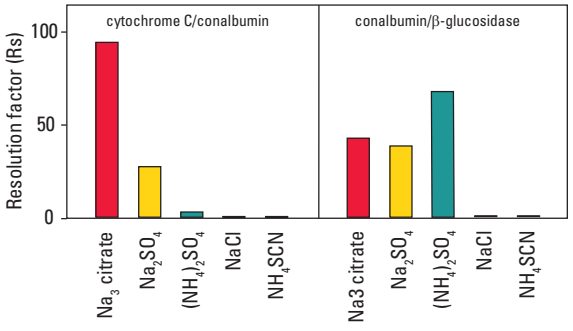
An optimum HIC process step will balance high dynamic binding capacity, adequate selectivity, good mass recovery and retention of biological activity. The wide range of selectivities for TOYOPEARL and TSKgel resins enables a developer to optimize protein separations at the extremes of the hydrophobic spectrum. The more hydrophobic ligands on TOYOPEARL Hexyl-type and TOYOPEARL Butyl-type resins are used to separate hydrophilic proteins. These two resins should also be considered for separations requiring a low salt environment.

TOYOPEARL and TSKgel Ether resins are used for the purification of very hydrophobic targets such as certain monoclonal antibodies and membrane proteins. These proteins may bind irreversibly to other more hydrophobic resins.

TOYOPEARL PPG and TOYOPEARL and TSKgel Phenyl resins complement the other HIC ligands available in the HIC series and offer alternatives for moderately hydrophobic proteins.

In addition to the hydrophobicity of the ligand, the selectivity in HIC is influenced by the eluent salt type. Figure 9 demonstrates the effect of salt type on the resolution factor of different protein pairs.[2] The Hofmeister lyotropic salt series shown in Figure 10 ranks anions and cations by their ability to promote protein precipitation. Ions on the left are referred to as "lyotropic" while the ions on the right are called "chaotropic". Lyotropic salts will precipitate or "salt out" proteins at high salt concentrations due to increased hydrophobic interaction, while chaotropic salts will promote protein denaturation at high salt concentrations. The Hofmeister lyotropic salt series indicates that the use of different salt systems may generate a variety of adsorption and desorption selectivities for each resin with a given protein. This feature of HIC provides an additional parameter for the optimization of a process step.

Figure 9: Influence of salt-type on resolution



Chromatography on a Toyopearl Butyl-substituted support

Resin:	**Toyopearl Butyl-650M**
Column size:	4.1 mm ID × 4 cm
Mobile phase:	Buffer A: 20 mmol/L phosphate buffer in 1.0 mol/L indicated salt, pH 7.0 Buffer B: buffer A with 1.0 mol/L indicated salt
Flow rate:	484 cm/h (1 mL/min)
Detection:	UV @ 280 nm

[2]Fausnaugh, J.; Kennedy, L.; Regnier, F. *J. Chromatogr.* **1984**, 141, 317.

Figure 10: Hofmeiseter lyotropic salt series

for anions

$SO_4^{2-} > HPO_4^{2-} > CH_3COO^- >$ halide $> NO_3^- > ClO_4^- > SCN^-$

for cations

$(CH_3)_4N^+ > K^+ > Na^+ > Cs^+ > Li^+ > Mg^{2+} > Ca^{2+} > Ba^{2+}$

Ammonium sulfate and sodium sulfate are the most commonly used salts in HIC. NaCl is often used as well.

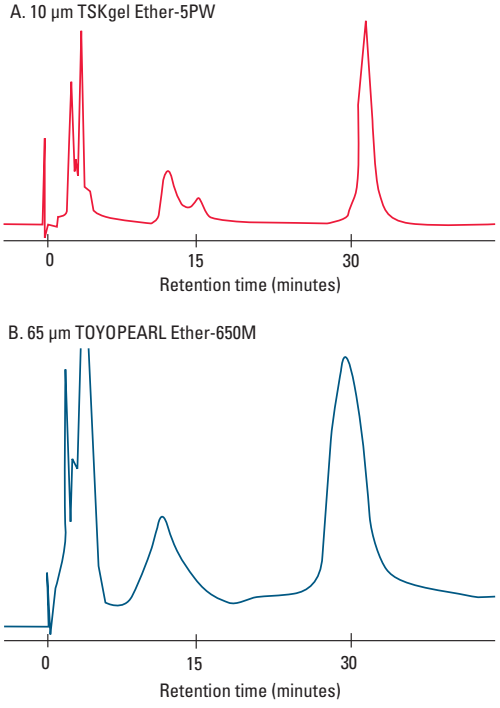
HIC is commonly used as a polishing step in monoclonal antibody purification processes. HIC offers an orthogonal selectivity to ion exchange chromatography and can be an effective step for aggregate clearance and host cell protein reduction, however, this mode of chromatography suffers from the limitation of use of high concentrations of kosmotropic salts to achieve the desired separation. Ghose et al[3] reports an unconventional way of operating HIC in the flowthrough (FT) mode with no kosmotropic salt in the mobile phase. TOYOPEARL Hexyl-650C was selected as the stationary phase and the pH of the mobile phase was modulated to achieve the required selectivity. Optimum pH conditions were chosen under which the antibody product of interest flowed through while impurities such as aggregates and host cell proteins bound to the column. The performance of the TOYOPEARL Hexyl-650C resin was comparable to that observed using conventional HIC conditions with high salt.

[3]Ghose, S.; Tao, Y.; Conley, L.; Cecchini, D. Purification of monoclonal antibodies by hydrophobic interaction chromatography under no-salt conditions. mAbs. 2013, 5, (5), 795-800.

Purification of Monoclonal Antibodies

For a very hydrophobic mAb, such as mouse anti-chicken lectin (14 kDa), the less hydrophobic TOYOPEARL Ether ligand works quite well. The purification of this mAb from ascites fluid (Figure 11) was performed with a 10 µm TSKgel Ether-5PW semi-preparative column. Identical selectivity for scale-up was found with corresponding 65 µm TOYOPEARL Ether-650M resin.

Figure 11: Purification of mAbs from ascites fluid

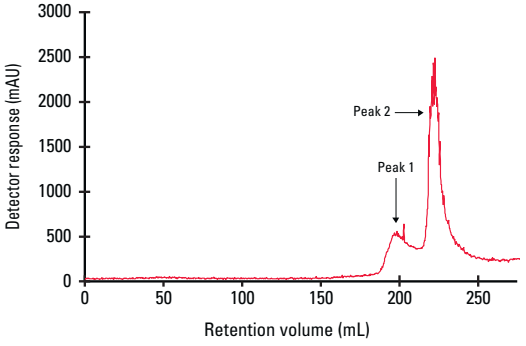


Resins:	**A. TSKgel Ether-5PW (prepacked HPLC column)** **B. TOYOPEARL Ether-650M**
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	Buffer A: 1.5 mol/L $(NH_4)_2SO_4$ + 0.1mol/L phosphate buffer, pH 7.0 Buffer B: 0.1 mol/L phosphate buffer, pH 7.0
Gradient:	60 min linear gradient from buffer A to B
Flow rate:	136 cm/h (1.0 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	A: 1.5 mg/100 µL anti-chicken 14 kDa lectin B: 0.76 mg/50 µL diluted ascites fluid
Load volume:	150 µL

Plasmid DNA Purification

TOYOPEARL Hexyl-650C resin was used successfully for plasmid DNA purification by Cambrex, Baltimore, MD (US patent 6,953,686). The resin was shown to be the most effective among HIC resins for endotoxin removal with capacities exceeding 2 million EU/mL of resin. Additionally, RNA and protein impurities were effectively eliminated. TOYOPEARL Hexyl-650C was also effective in separating the supercoiled and open circular forms of plasmid DNA (Figure 12). Under certain binding conditions, the two forms are bound to the resin, and subsequently eluted with a simple gradient, resulting in two distinct peaks corresponding to the relaxed and supercoiled forms respectively.

Figure 12: Plasmid DNA separation

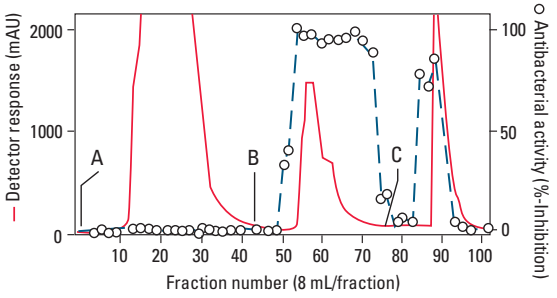


Resin:	**TOYOPEARL Hexyl-650C**
Column size:	1.0 cm ID × 30 cm (23.6 mL)
Mobile phase:	Buffer A. 3.0 mol/L ammonium sulfate, 10 mmol/L Tris-HCl buffer, 1 mmol/L EDTA, pH 7.4 Buffer B. 10 mmol/L Tris-HCl buffer, 1 mmol/L EDTA, pH 7.4
Gradient:	3.0 mol/L ammonium sulfate to 1.0 mol/L ammonium sulfate (6 CV)
Flow rate:	153 cm/h (2.0 mL/min)
Detection:	UV @ 254 nm
Sample:	Plasmid DNA in 3.0 mol/L ammonium sulfate

Purification of Glycoproteins

TOYOPEARL HIC resins can purify glycoproteins, which often bind irreversibly to saccharide-based chromatographic media. Figure 13 shows the purification of a large glycoprotein on TOYOPEARL Butyl-650S resin.

Figure 13: Large glycoprotein purified on TOYOPEARL Butyl-650S

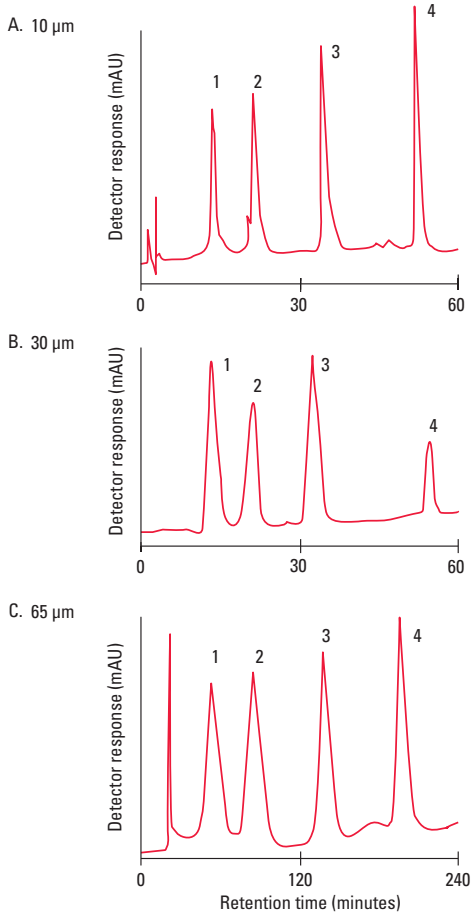


Resin:	**Toyopearl Butyl-650S**
Column size:	22 mm ID × 26 cm
Mobile phase:	Buffer A: 40 % saturated $(NH_4)_2SO_4$ + 50 mol/L phosphate buffer, pH 7.0 Buffer B: 50 mol/L phosphate buffer, pH 7.0
Gradient:	A: Load and wash in 100 % buffer A B: 50 % buffer A with 50 % buffer B C: 100 % buffer B
Detection:	UV @ 280 nm
Sample:	crude protein from sea hare *Aplysia kurodai*

Ultra Purification of Target Compound

Biopharmaceutical process development often requires a high performance step for ultra-purification of a target compound. To meet these needs, 20 and 30 µm TSKgel Phenyl-5PW and Ether-5PW are available. The selectivity of these packings is similar to the 10 µm TSKgel 5PW Phenyl-5PW and Ether-5PW analytical columns. Therefore methods can easily be transferred from analytical to preparative scale resins of the same chemistry using a seamless scale-up strategy. Figure 14 shows the similar elution pattern on 10 µm and 30 µm TSKgel packings, along with 65 µm TOYOPEARL process-scale resin.

Figure 14: Seamless scale up

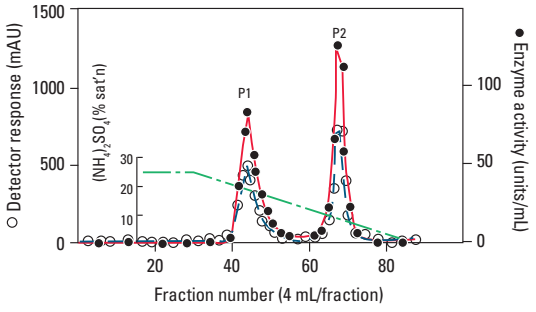


Resins:	**A and B. TSKgel Phenyl-5PW** **C. TOYOPEARL Phenyl-650M**
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	A and B: 60 min linear gradient from 1.8 mol/L to
Gradient:	0 mol/L $(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer C: 240 min linear gradient from 1.8 mol/L to 0 mol/L$(NH_4)_2SO_4$ in 0.1 mol/L phosphate buffer
Flow rate:	A and B. 68 cm/hr (0.50 mL/min) C. 272 cm/h (2.0 mL/min)
Detection:	UV @ 280 nm
Samples:	1. myoglobin 2. ribonuclease A 3. lysozyme 4. α-chymotrypsinogen
Load volume:	100 µL containing 1 g/L of each protein

Purification and Resolution of Pullulanase

The power of HIC is illustrated in a scheme in which pullulanase, an amylase-like enzyme responsible for hydrolysis of branched chain sugars, is purified and resolved into two closely related forms. Ion exchange and size exclusion chromatography effectively purified pullulanase. With TOYOPEARL Butyl-650S, however, two closely related proteins were resolved, based on differences in their surface hydrophobicity (Figure 15).

Figure 15: Separation of two active pullulanase forms

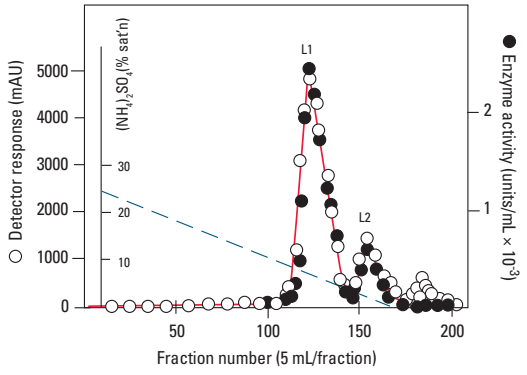


Resin:	**TOYOPEARL Butyl-650S**
Column size:	18 mm ID × 16 cm
Mobile phase:	isocratic elution, 120 mL $(NH_4)_2SO_4$, 25 % saturation in 0.02 mol/L phosphate buffer, pH 7.0, followed by a linear gradient, 224 mL $(NH_4)_2SO_4$, 25 % to 0 % saturation, in buffer
Flow rate:	12 cm/h
Detection:	UV @ 280 nm
Sample:	protein from *Bacillus acidopullulyticus*
Sample load:	20 mg

Lipase Isozymes

Incorporation of HIC into a purification scheme has separated lipase isozymes that were not resolved by a previously reported method. After ion exchange and size exclusion chromatography, an additional step employing TOYOPEARL Butyl-650M, as shown in Figure 16, enabled the separation of two active lipase isozymes, L1 and L2, from an inactive impurity. Activity recovery was 93 % for this step.

Figure 16: Separation of lipase isozymes from impurity



Resin:	**TOYOPEARL Butyl-650M**
Column size:	34 mm ID × 29 cm
Mobile phase:	linear gradient, 810 mL $(NH_4)_2SO_4$, 25 % to 0 % saturation in 0.01 mol/L acetate buffer, pH 5.6
Flow rate:	2 cm/h
Detection:	UV @ 254nm
Sample:	lipase from *Geotrichum candidum*
Sample load:	375 mg

A selection of screening tools are available for TOYOPEARL and TSKgel HIC resins. See the Process Development Products section of this Product Guide for details.

Ordering Information

TOYOPEARL HIC resins:

Part #	Product description	Container size (mL)	Bead diameter (µm)	Typical lysozyme capacity (g/L)
0043151	TOYOPEARL Ether-650S	25	20 - 50	10 - 30
0016172	TOYOPEARL Ether-650S	100		
0016174	TOYOPEARL Ether-650S	1,000		
0016176	TOYOPEARL Ether-650S	5,000		
0019805	TOYOPEARL Ether-650M	25	40 - 90	10 - 30
0016173	TOYOPEARL Ether-650M	100		
0016175	TOYOPEARL Ether-650M	1,000		
0016177	TOYOPEARL Ether-650M	5,000		
0021301	TOYOPEARL PPG-600M	25	40 - 90	45 - 55
0021302	TOYOPEARL PPG-600M	100		
0021303	TOYOPEARL PPG-600M	1,000		
0021304	TOYOPEARL PPG-600M	5,000		
0021305	TOYOPEARL PPG-600M	50,000		
0021887	TOYOPEARL Phenyl-600M	25	40 - 90	45 - 65
0021888	TOYOPEARL Phenyl-600M	100		
0021889	TOYOPEARL Phenyl-600M	1,000		
0021890	TOYOPEARL Phenyl-600M	5,000		
0020891	TOYOPEARL Phenyl-600M	50,000		
0043152	TOYOPEARL Phenyl-650S	25	20 - 50	30 - 50
0014477	TOYOPEARL Phenyl-650S	100		
0014784	TOYOPEARL Phenyl-650S	1,000		
0014935	TOYOPEARL Phenyl-650S	5,000		
0019818	TOYOPEARL Phenyl-650M	25	40 - 90	30 - 50
0014478	TOYOPEARL Phenyl-650M	100		
0014783	TOYOPEARL Phenyl-650M	1,000		
0014943	TOYOPEARL Phenyl-650M	5,000		
0018364	TOYOPEARL Phenyl-650M	50,000		
0043126	TOYOPEARL Phenyl-650C	25	50 - 150	30 - 50
0014479	TOYOPEARL Phenyl-650C	100		
0014785	TOYOPEARL Phenyl-650C	1,000		
0014944	TOYOPEARL Phenyl-650C	5,000		

Part #	Product description	Container size (mL)	Bead diameter (µm)	Typical lysozyme capacity (g/L)
0043153	TOYOPEARL Butyl-650S	25	20 - 50	30 - 50
0007476	TOYOPEARL Butyl-650S	100		
0014701	TOYOPEARL Butyl-650S	1,000		
0007975	TOYOPEARL Butyl-650S	5,000		
0018826	TOYOPEARL Butyl-650S	50,000		
0019802	TOYOPEARL Butyl-650M	25	40 - 90	30 - 50
0007477	TOYOPEARL Butyl-650M	100		
0014702	TOYOPEARL Butyl-650M	1,000		
0007976	TOYOPEARL Butyl-650M	5,000		
0018355	TOYOPEARL Butyl-650M	50,000		
0043127	TOYOPEARL Butyl-650C	25	50 - 150	30 - 50
0007478	TOYOPEARL Butyl-650C	100		
0014703	TOYOPEARL Butyl-650C	1,000		
0007977	TOYOPEARL Butyl-650C	5,000		
0022826	TOYOPEARL Butyl-650C	50,000		
0021448	TOYOPEARL Butyl-600M	25	40 - 90	40 - 60 γ-globulin
0021449	TOYOPEARL Butyl-600M	100		
0021450	TOYOPEARL Butyl-600M	1,000		
0021451	TOYOPEARL Butyl-600M	5,000		
0021452	TOYOPEARL Butyl-600M	50,000		
0019955	TOYOPEARL SuperButyl-550C	25	50 - 150	52 - 70
0019956	TOYOPEARL SuperButyl-550C	100		
0019957	TOYOPEARL SuperButyl-550C	1,000		
0019958	TOYOPEARL SuperButyl-550C	5,000		
0019959	TOYOPEARL SuperButyl-550C	50,000		
0044465	TOYOPEARL Hexyl-650C	25	50 - 150	30 - 50
0019026	TOYOPEARL Hexyl-650C	100		
0019027	TOYOPEARL Hexyl-650C	1,000		
0019028	TOYOPEARL Hexyl-650C	5,000		
0021973	TOYOPEARL Hexyl-650C	50,000		

TSKgel HIC resins:

Part #	Product description	Container size (mL)	Bead diameter (µm)	Typical lysozyme capacity (g/L)
0043276	TSKgel Ether-5PW (20)	25	15 - 25	10 - 30
0016052	TSKgel Ether-5PW (20)	250		
0016053	TSKgel Ether-5PW (20)	1,000		
0018437	TSKgel Ether-5PW (20)	5,000		
0043176	TSKgel Ether-5PW (30)	25	20 - 40	10 - 30
0016050	TSKgel Ether-5PW (30)	250		
0016051	TSKgel Ether-5PW (30)	1,000		
0018439	TSKgel Ether-5PW (30)	5,000		
0043277	TSKgel Phenyl-5PW (20)	25	15 - 25	15 - 35
0014718	TSKgel Phenyl-5PW (20)	250		
0014719	TSKgel Phenyl-5PW (20)	1,000		
0018438	TSKgel Phenyl-5PW (20)	5,000		
0043177	TSKgel Phenyl-5PW (30)	25	20- 40	10 - 30
0014720	TSKgel Phenyl-5PW (30)	250		
0014721	TSKgel Phenyl-5PW (30)	1,000		
0017210	TSKgel Phenyl-5PW (30)	5,000		


TOYOPEARL®
Phenyl-650M
Net Vol. 100 mL
Part No. 14478
Lot No.
FOR IN VITRO USE ONLY
TOYOPEARL®
Butyl-650M
25 mL
19802
TSKgel®
Phenyl-5PW (20)
25 mL
43277

TOYOPEARL AF-rProtein A-650F

TOYOPEARL AF-rProtein A HC-650F

Protein A Chromatography in Process Purification

Protein A chromatography, the most widely used type of affinity chromatography, relies on the specific and reversible binding of antibodies to an immobilized ligand; in this case protein A. Protein A is a 56 kDa surface protein native to the cell wall of the bacterium *Staphylococcus aureus*. It is composed of five immunoglobulin-binding domains, each of which are able to bind proteins from many mammalian species, most notably Immunoglobulin G (IgG) through the heavy chain within the Fc region. While the native form of protein A was used as the ligand for first generation protein A resins, the recombinant form (rProtein A) produced in *E. coli* is the most prevalent today. Modifications to the protein structure of the ligand, the advent of ligands composed of single domain multimers, and multipoint attachment have given rise to the caustic stable, high capacity and extremely robust protein A resins in use today.

The protein A ligand can either bind directly to the Fc region of an antibody or to an Fc tag that has been fused to the target of interest. Protein A chromatography is a very robust purification procedure and is used as a capture step due to its specificity and, depending on the intended use for the target molecule (antibodies for diagnostic testing), might be the only chromatographic step required to achieve adequate product purity.

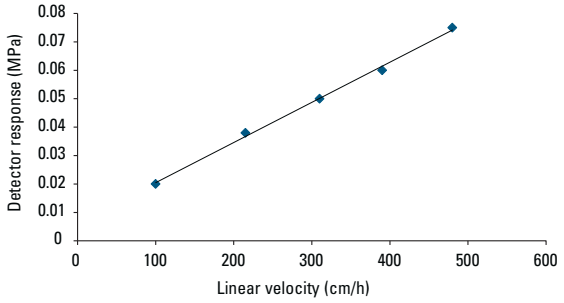
In protein A chromatography, crude feed stock is passed through a column under conditions that promote binding. After loading is complete, the column is washed under conditions that do not interrupt the specific interaction between the target and ligand, but that will disrupt any non-specific interactions between process impurities (host cell proteins, etc.) and the stationary phase. The bound protein is then eluted with mobile phase conditions that disrupt the target/ligand interactions. Elution of the target molecule from protein A resin is most commonly accomplished by lowering the pH of the mobile phase, creating an environment whereby the structure of the target molecule is altered in such a way as to inhibit binding. Low pH elution can have a negative effect on protein stability and it is advised that the eluted protein solution be neutralized to minimize aggregation and denaturation.

TOYOPEARL Protein A Resins

Tosoh Bioscience offers two TOYOPEARL affinity resins with a recombinant protein A ligand. TOYOPEARL AF-rProtein A resins are composed of hydrophilic, dimensionally stable base resins that exhibit excellent pressure-flow characteristics. These resins use the TOYOPEARL HW-65 SEC resin as a base bead. The 100 nm pore diameter of the TOYOPEARL affinity resins can accommodate large globular proteins up to 5×10^6.

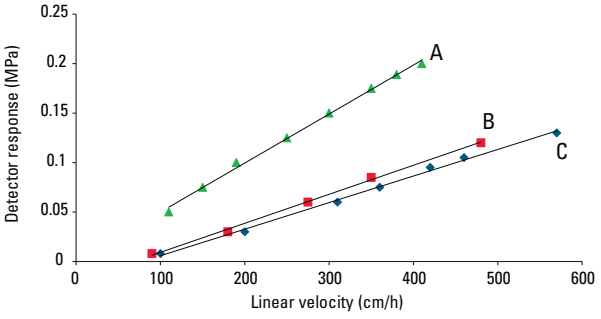
TOYOPEARL AF-rProtein A resins remain dimensionally stable within wide extremes of pH and ionic strength. Moreover, the semi-rigid TOYOPEARL AF-rProtein A particles do not distort under flow rates that generate up to 0.3 MPa pressure. These properties of the resins, combined with the narrow particle size distributions, result in superior pressure-flow characteristics for the packed TOYOPEARL bed. Linear velocities of 300 – 500 cm/h generate a pressure of between 0.1 and 0.2 MPa in a packed bed (Figures 1A and 1B).

Figure 1A: Linear velocity and pressure curve



Resin:	**TOYOPEARL AF-rProtein A -650F**
Column and size:	Resolute®, 40 cm ID × 8.4 cm
Mobile phase:	water
Linear velocity:	various
Detection:	pressure (MPa)

Figure 1B: Comparison of linear velocity and pressure curves



Resins:	**A. TOYOPEARL AF-rProtein A -650F, 45 µm, 20 cm ID × 32 cm** **B. TOYOPEARL AF-rProtein A -650F, 45 µm, 20 cm ID × 18 cm** **C. TOYOPEARL Butyl-650M, 65 µm, 20 cm ID × 21 cm**
Column:	QuikScale®, 20 cm ID
Mobile phase:	water
Linear velocity:	various
Detection:	pressure (MPa)

TOYOPEARL resin	Functionality	Base bead	Pore size	Bead diameter	Ligand type	Ligand leakage	DBC (g/L)	Pressure rating
AF-rProtein A-650F	Protein A	HW-65	100 nm	45 µm	rProtein A	5 - 25 µg/g	> 30 @ 3 min	0.3 MPa
AF-rProtein A HC-650F	Protein A	HW-65	100 nm	45 µm	rProtein A	0.6 - 1.7 µg/g	> 65 @ 5 min	0.3 MPa

TOYOPEARL AF-rProtein A HC-650F resin is the newest affinity resin to be introduced from Tosoh Bioscience. This new, high capacity protein A resin was introduced in the Fall of 2013. An enhanced rProtein A ligand (Figure 2) is bound to the TOYOPEARL HW-65F base bead via multipoint attachment resulting in excellent base (Figure 3) stability for up to 200 CIP cycle with 0.1 mol/L NaOH. TOYOPEARL AF-rProtein A HC-650F resin maintains 80 % of initial dynamic binding capacity after 40 CIP cycles with 0.5 mol/L NaOH (Figure 4). TOYOPEARL AF-rProtein A HC-650F resin exhibits dynamic binding capacities of greater than 65 g/L at residence times of 5 minutes and greater than 50 g/L at 2 minutes residence time with feed stock concentrations from 1.0 g/L to 10.0 g/L (Figure 5).

Figure 2: Ligand structure of TOYOPEARL AF-rProtein A HC-650F resin

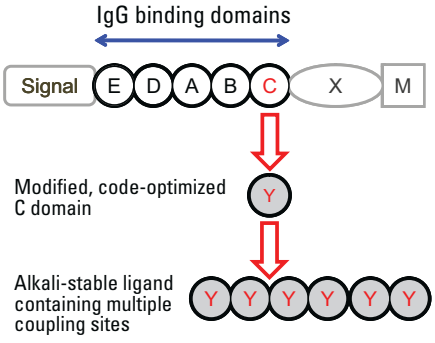
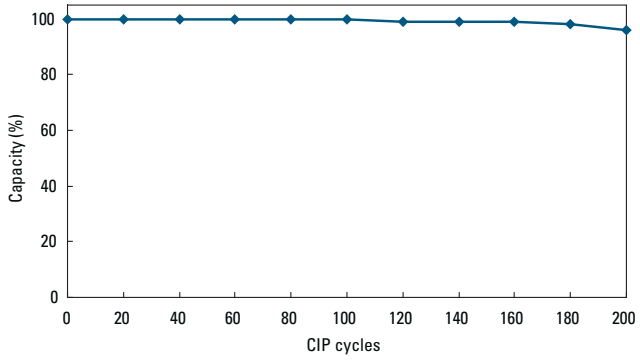
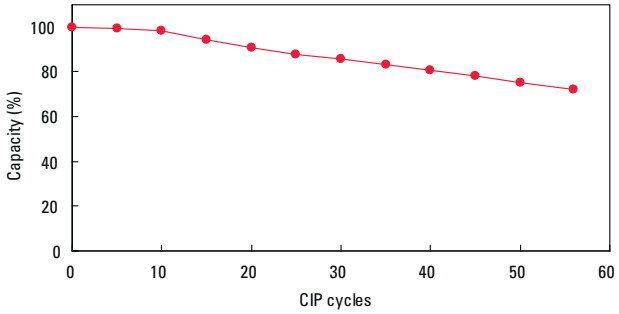


Figure 3: Base stablity of TOYOPEARL AF-rProtein A HC-650F



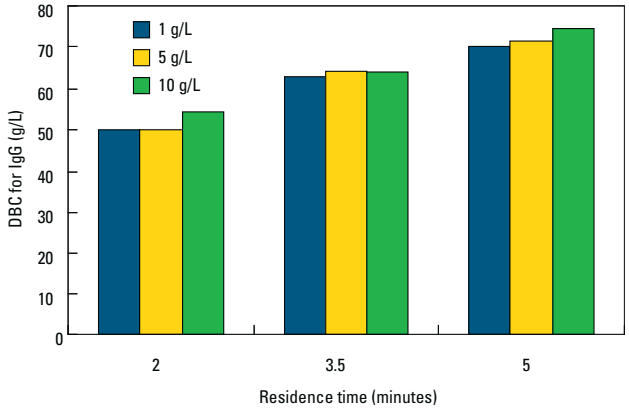
Column size:	5 mm ID × 5 cm
Wash procedure:	A: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (10 CV) B: 0.1 mol/L citrate buffer, pH 3.0 (5CV) C: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (7 CV) D: 0.1 mol/L NaOH (3 CV – 15 min contact time) E: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (5 CV)
Capacity:	DBC was determined at 10 % breakthrough after every 20 cycles

Figure 4: DBC of TOYOPEARL AF-rProtein A HC-650F resin after CIP with 0.5 mol/L NaOH



Column size:	5 mm ID × 5 cm
Wash procedure:	A: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (10 CV) B: 0.1 mol/L citrate buffer, pH 3.0 (5 CV) C: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (7 CV) D: 0.5 mol/L NaOH (3 CV – 15 min contact time) E: 20 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4 (5 CV)
Capacity:	DBC was determined at 10 % breakthrough after every 5 cycles

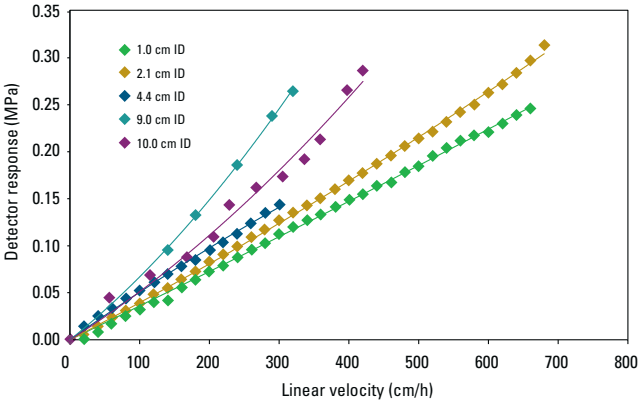
Figure 5: DBC of of TOYOPEARL AF-rProtein A HC-650F



Resin:	**TOYOPEARL AF-rProtein A HC-650F**
Column size:	5 mm ID × 5 cm
Mobile phase:	0.02 mol/L sodium phosphate buffer, 0.15 mol/L NaCl, pH 7.4
Residence time:	2, 3.5, 5 min
Detection:	UV @ 280 nm (10 % breakthrough)
Sample:	human IgG @ 1, 5, 10 g/L in mobile phase

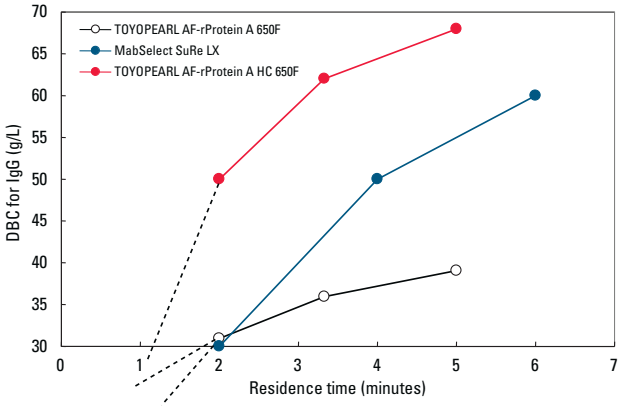
Achievement of high linear velocities at relatively low pressure enables high throughput at production scale using equipment with moderate pressure limitations (Figure 6). Improved mass transfer characteristics allow it to maintain a larger percent of its capacity at lower residence times (Figure 7) relative to agarose base stable resins. Typical leakage for this rProtein A ligand is 0.6 -1.7 ng rProtein A / mg eluted antibody by ELISA testing (Table 1). Though TOYOPEARL AF-rProtein A-650F resin is shipped in 20 % ethanol, it can be stored in 2 % benzyl alcohol if necessary.

Figure 6: Comparison of linear velocity and pressure curves



Column sizes:	1.0 cm ID, 2.1 cm ID, 4.4 cm ID, 9.0 cm ID, 10.0 cm ID 20 cm normalized bed height
Mobile phase:	deionized water
Detection:	pressure (MPa)

Figure 7: Comparison of residence time and capacity



Resins:	**TOYOPEARL AF-rProtein A HC-650F** **TOYOPEARL AF-rProtein A-650F** MabSelect SuRe™ LX
Column size:	5 mm ID ×5 cm
Mobile phase:	0.02 mol/L sodium phosphate buffer, 0.15 mol/L NaCl, pH 7.4
Residence time*:	2, 3.5, 5 min
Detection:	UV @ 280 nm
Sample:	human IgG @ 1 g/L in mobile phase

*MabSelect SuRe DBC data taken from product brochure(2, 4, and 6 minute residence times).

DBC was calculated at 10 % breakthrough

Table 1: Ligand leakage before and after CIP

Amount of ligand leakage (ppm)	Before CIP		After 200 CIP cycles	
	Elution Buffer		Elution Buffer	
	citrate (pH 3.0)	glycine-HCl (pH 3.0)	citrate (pH 3.0)	glycine-HCl (pH 3.0)
	1.7	1.6	0.6	0.5
Amount of ligand leakage was determined with TOYOPEARL AF-rProtein A HC-650F ELISA				
ppm = ng/mg IgG				

TOYOPEARL AF-rProtein A-650F resin is an affinity resin for monoclonal antibody purification. The recombinant ligand (Figure 8) is expressed in *E. coli* and is free of animal derived products. The ligand is bound to the TOYOPEARL HW-65F base bead via multipoint attachment resulting in excellent base (Figure 9 and 10) and thermal stability (Figure 11). TOYOPEARL AF-rProtein A-650F resin exhibits dynamic binding capacities of greater than 30 g/L at residence times of 3 minutes and greater.

Figure 8: Ligand structure of TOYOPEARL AF-rProtein A-650F resin

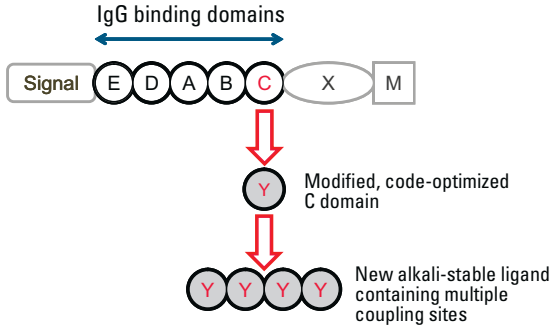
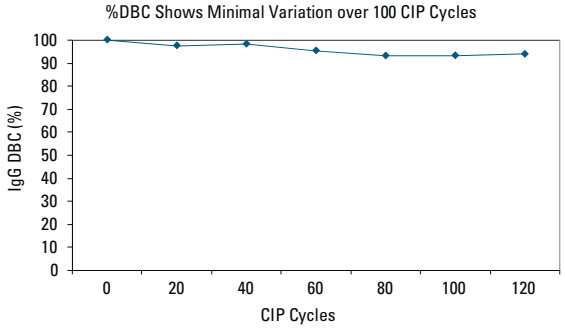


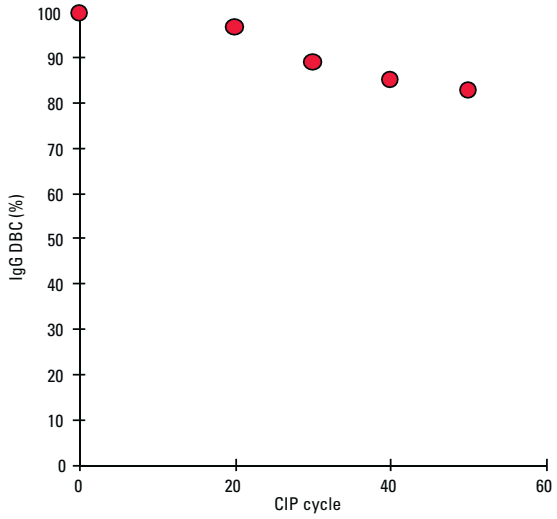
Figure 9: CIP stability to 0.1 mol/L HaOH



Resin:	**TOYOPEARL AF-rProtein A-650F**
Column size:	4.6 mm ID × 10 cm
Mobile phase:	Buffer A: 0.15 mol/L NaCl in 0.02 mol/L sodium phosphate buffer, pH 7.2 Buffer B: 0.10 mol/L citrate buffer, pH 3.0
Cycle volumes:	5 column volumes buffer A 10 column volumes buffer B 3 column volumes 0.1 mol/L NaOH, (16 min contact time) 5 column volumes H_2O

DBC was calculated at 10 % breakthrough

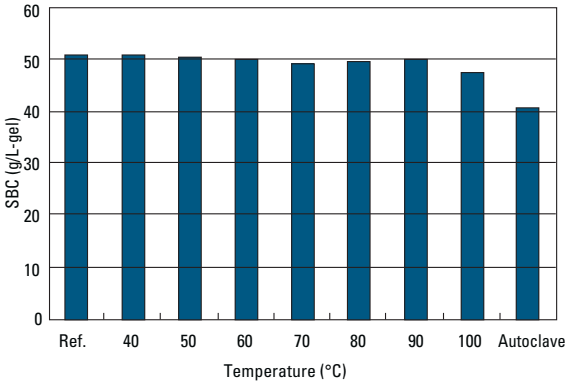
Figure 10: CIP stability to 0.5 mol/L NaOH



Resin:	**TOYOPEARL AF-rProtein A-650F**
Column size:	5.0 mm ID × 5 cm
Mobile phase:	Buffer A: 0.15 mol/L NaCl in 0.02 mol/L sodium phosphate buffer, pH 7.2 Buffer B: 0.10 mol/L citrate buffer, pH 3.0
Cycle volumes:	5 column volumes binding buffer 10 column volumes elution buffer 8 column volumes 0.5 mol/L NaOH, (16 min contact time) 5 column volumes H_2O

DBC was calculated at 10 % breakthrough

Figure 11: Temperature stability



Resin:	**TOYOPEARL AF-rProtein A-650F**
Mobile phase:	deionized water
Autoclave settings:	120 °C, 1.2 atm, 15 min
Heating time:	30 min

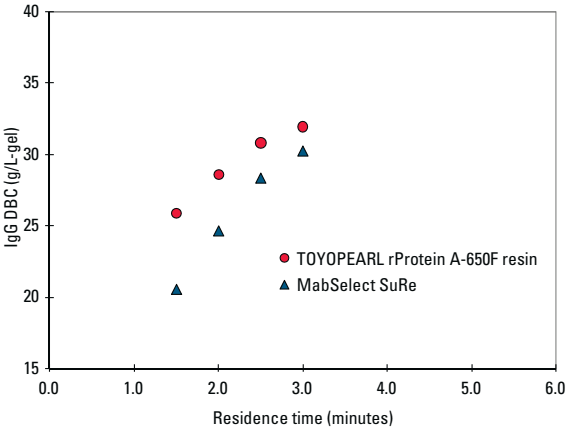
TOYOPEARL AF-rProtein is stable at 35 °C for least 3 years (data not shown)

Improved mass transfer characteristics allow TOYOPEARL AF-rProtein A-650F to maintain a larger percent of its capacity at lower residence times (Figure 12) relative to agarose base stable resins. Typical leakage for this rProtein A ligand is 5-25 ng rProtein A /mg eluted antibody by ELISA testing.

Achievement of high linear velocities at relatively low pressure enables high throughput at production scale using equipment with moderate pressure limitations. Sanitization or cleaning may be conducted with up to 0.5 mol/L NaOH or 0.5 mol/L HCl depending upon the ligand.

An important aspect of the use of a Protein A resin in the capture step is its ability to remove host cell protein (HCP) from the feedstock. TOYOPEARL AF-rProtein A-650F addresses this key area as well (Table 2).

Figure 12: DBC at various residence times



Resins:	**TOYOPEARL AF-rProtein A-650F**
	MabSelect SuRe
Column size:	5 mm ID × 5 cm (1 mL)
Mobile phase:	0.02 mol/L sodium phosphate buffer, pH 7.2 + 0.15 mol/L NaCL
Residence time:	1.5, 2.0, 2.5, 3.0 min

Table 2: TOYOPEARL AF-rProtein A-650F resin vs. MabSelect SuRe resin

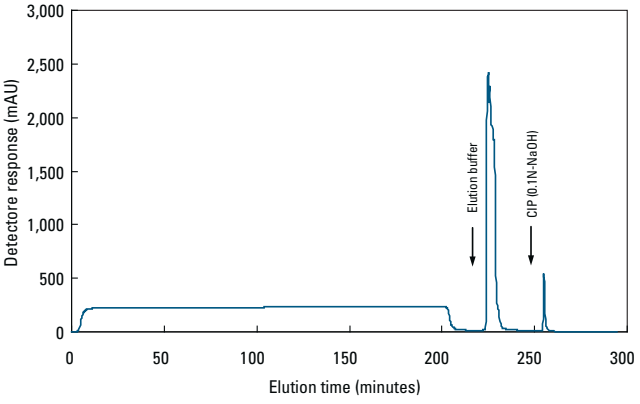
Resin	Protein load (g/L gel)	pH	Flow (cm/h)	BV (µL)	Buffer	CHO (µg/L)
Toyopearl AF-rProtein A-650F MabSelect SuRe	5 5	3,9 3,9	250 250	200 200	Tris Phosphate	9,76 30,52
Toyopearl AF-rProtein A-650F MabSelect SuRe	45 45	3,4 3,4	100 100	200 200	Tris Phosphate	0,67 36,52
Toyopearl AF-rProtein A-650F MabSelect SuRe	25 25	3,9 3,9	250 250	200 200	Tris Phosphate	47,26 >310
Toyopearl AF-rProtein A-650F MabSelect SuRe	5 5	3,9 3,9	100 100	200 200	Tris Phosphate	19,16 81,32

Data kindly provided by U. Breuninger, University of Applied Science Esslingen. Both resins were packed in Media Scout® Columns, Atoll GmbH, Weingarten.

Purification of Monoclonal Antibodies

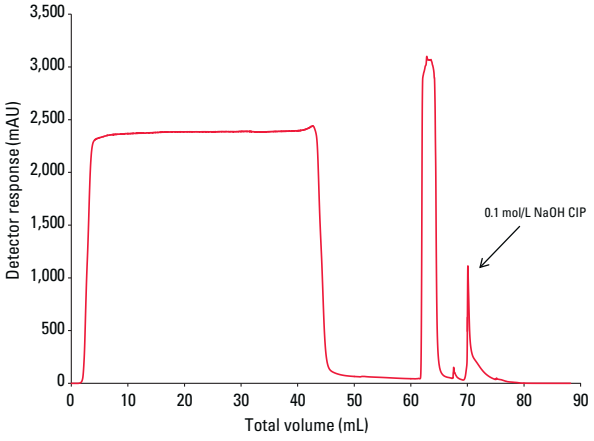
TOYOPEARL AF-rProtein A HC-650F was used for the purification of a monoclonal antibody from CHO cell culture supernatant with a concentration of 1.0 g/L (Figure 13) at 5 minutes residence time in a 5 cm bed height column. As can be seen from the chromatogram, tailing is minimal on the elution peak and the eluted mAb is > 95 % pure by SEC. A second purification using a 9.7 cm bed height with a feedstock concentration of 2.9 g/L and 5 minute residence time can be seen in Figure 14.

Figure 13: Purification of monoclonal antibody



Resin:	**TOYOPEARL Protein A**
Column size:	5 mm ID × 5.0 cm
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate buffer containing 0.15 mol/L NaCl, pH 7.4
	Buffer B: 0.1 mol/L citrate buffer, pH 3.0
Flow rate:	61 cm/h (0.2 mL/min)
Residence time:	5 min
Sample:	40 mL of CHO cell culture, containing 1.0 gL humanized IgG_1

Figure 14: Second purification of monoclonal antibody



Resin:	**TOYOPEARL AF-rProtein A HC-650F**
Column size:	5.0 mm ID × 9.7 cm (1.90 mL)
Mobile phase:	Buffer A: 20 mmol/L Na_2HPO_4, 150 mmol/L NaCl, pH 7.39
	Buffer B: 100 mmol/L Na citrate, pH 3.0
Flow rate:	116 cm/h (0.38 mL/min)
Residence time:	5 min
Detection:	UV @ 280 nm
Sample:	Crude feedstock (mAb)

A selection of screening tools are available for TOYOPEARL Protein A resins. See the Process Development Products section of this Product Guide for details.

Ordering Information

TOYOPEARL Protein A resins:

Part #	Product description	Container size (mL)
0022803	TOYOPEARL AF-rProtein A-650F	10
0022804	TOYOPEARL AF-rProtein A-650F	25
0022805	TOYOPEARL AF-rProtein A-650F	100
0022806	TOYOPEARL AF-rProtein A-650F	1,000
0022807	TOYOPEARL AF-rProtein A-650F	5,000
0022808	TOYOPEARL AF-rProtein A-650F	50,000
0022815	TOYOPEARL AF-rProtein A-650F ELISA*	
0023425	TOYOPEARL AF-rProtein A HC-650F	10
0023426	TOYOPEARL AF-rProtein A HC-650F	25
0023427	TOYOPEARL AF-rProtein A HC-650F	100
0023428	TOYOPEARL AF-rProtein A HC-650F	1,000
0023429	TOYOPEARL AF-rProtein A HC-650F	5,000
0023434	TOYOPEARL AF-rProtein A HC-650F	50,000
0023433	TOYOPEARL AF-rProtein A HC-650F ELISA**	

* This kit is specifically prepared for TOYOPEARL AF-rProtein A-650F resin. Test kits for other commercially available Protein A products may not work properly for TOYOPEARL AF-rProtein A-650F resin.

** This kit is specifically prepared for TOYOPEARL AF-rProtein A HC-650F resin. Test kits for other commercially available Protein A products may not work properly for TOYOPEARL AF-rProtein A HC-650F resin.

Resin Type	Process Media
Activated Resins	TOYOPEARL AF-Epoxy-650 TOYOPEARL AF-Tresyl-650
Reactive Resins	TOYOPEARL AF-Carboxy-650 TOYOPEARL AF-Amino-650 TOYOPEARL AF-Formyl-650
Ready-to-Use Resins with Group Specific Ligands	TOYOPEARL AF-Blue HC-650 TOYOPEARL AF-Chelate-650 TOYOPEARL AF-Red-650 TOYOPEARL AF-Heparin HC-650

The role of Affinity Chromatography in Process Purification

Affinity chromatography (AFC) is distinctive as a chromatographic technique as it is the only mode that enables the purification of a biomolecule on the basis of functionality or unique chemical structure. Affinity chromatography works on the basis of a specific and reversible interaction between a target molecule and a specific ligand coupled to a base chromatography bead. Affinity chromatography is inherently a high resolution media because it is highly selective for the molecule of interest, and usually high capacity as well. Because affinity resins are highly specific, post-purification product titer increases of more than 1000× are possible. Purifications that would otherwise be difficult or even impractical using other modes can often be easily accomplished through the use of affinity chromatography.

TOYOPEARL Affinity Resins

There are many custom designed affinity ligands available to the chromatographer. TOYOPEARL affinity chromatography resins are functionalized with chemically active groups or with group-specific ligands. Resins with activated functional groups are ready to directly couple a protein or other ligand. The coupled ligand must preserve its specific binding affinity for the target molecule and the binding between the ligand and target molecule must be reversible to allow the target to be eluted without substantial alterations. Resins with reactive groups require carbodiimide coupling or reductive amination to achieve a stable covalent linkage.

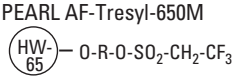
Tosoh Bioscience offers a spectrum of carefully selected TOYOPEARL affinity resins with activated or reactive groups which can be used to covalently attach almost any custom ligand. The structures of TOYOPEARL resins with activated and reactive ligands are shown in Figure 1 and structures of TOYOPEARL resins with group-specific ligands are listed in Figure 2. In general, TOYOPEARL AF-Tresyl-650M and TOYOPEARL AF-Formyl-650M resins are recommended for coupling proteins, while TOYOPEARL AF-Epoxy-650M resin is suited for coupling lower molecular weight ligands. TOYOPEARL AF-Amino-650M and TOYOPEARL AF-Carboxy-650M resins may be used for both. TOYOPEARL affinity resins may also be used in combinatorial chemistry or for solid phase synthesis of peptides and oligonucleotides because of their excellent stability in a variety of organic solvents and under extremes of pH.

TOYOPEARL affinity resins are composed of hydrophilic, dimensionally stable base resins that exhibit excellent pressure-flow characteristics. These resins use the TOYOPEARL HW-65 SEC resin as a base bead. The 100 nm pore diameter of the TOYOPEARL affinity resins can accommodate large globular proteins up to 5×10^6.

Figure 1: Activated and reactive TOYOPEARL affinity resins

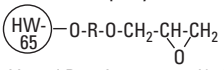
Activated TOYOPEARL affinity resins

TOYOPEARL AF-Tresyl-650M



Ligand Density: 80 µmol/g (dry)

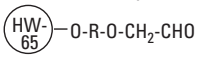
TOYOPEARL AF-Epoxy-650M



Ligand Density: 800 µmol/g (dry)

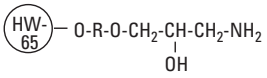
Reactive TOYOPEARL affinity resins

TOYOPEARL AF-Formyl-650M



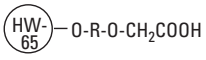
Ligand Density: 60 µeq/mL

TOYOPEARL AF-Amino-650M



Ligand Density: 100 µmol/mL

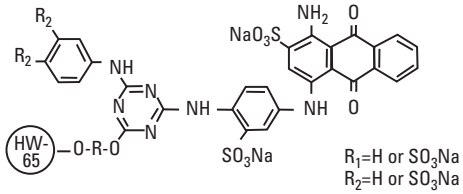
TOYOPEARL AF-Carboxy-650M



Ligand Density: 100 eq/mL

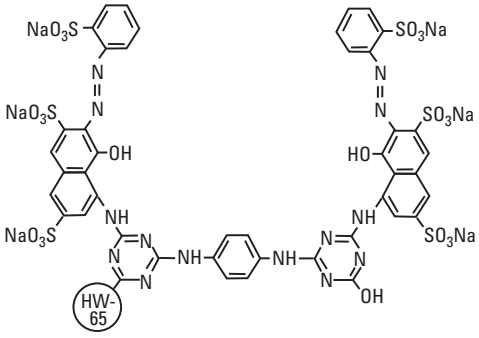
Figure 2: Group-specific TOYOPEARL affinity resins

TOYOPEARL AF-Blue HC

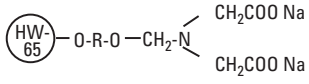


Ligand Density: 15 µmol/mL

TOYOPEARL AF-Red

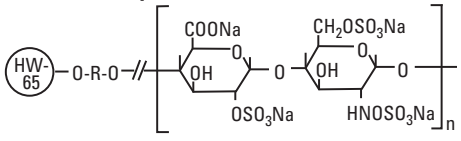


TOYOPEARL AF-Chelate



Ligand Density: 20 µmol/mL

TOYOPEARL AF-Heparin

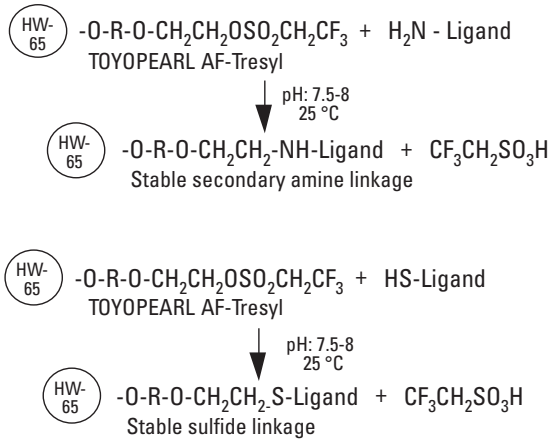


Approximate Ligand Density: 5 mg/mL

Activated resins – ready for direct ligand attachment

TOYOPEARL AF-Tresyl-650M activated resin is highly reactive toward amine and thiol groups. It is provided in dry form, ready for reaction in buffered solutions containing the ligand to be coupled. Coupling is accomplished in a neutral to slightly alkaline (pH 7 - 8) solution (Figure 3).

Figure 3: Coupling procedure for TOYOPEARL AF-Tresyl-650M



R = hydrophilic polymer

Under such conditions even proteins of limited stability may be successfully coupled. Coupling leads to the formation of a highly stable secondary amine or thio-ether linkage. The optimized tresyl density (ca. 20 µmol/mL hydrated resin) is sufficient to provide substantial protein binding while avoiding excessive multi-point attachment and consequent impairment of ligand affinity and activity. Representative data are presented in Table 1.

TOYOPEARL AF-Epoxy-650M activated resin, also packaged in dry form, has a high density of epoxy-functionality (ca. 800 µmol/mL). Under appropriate reaction conditions, this may be used to immobilize proteins or low molecular weight ligands. It is particularly useful when high densities of low molecular weight ligands must be attached (Figure 4). Glutathione and glycine have, for example, been coupled at densities greater than 100 µmol/mL hydrated resin. TOYOPEARL AF-Epoxy-650M resin is a highly versatile starting material for conversion to other chemically active functional groups required in special applications. This resin may be readily activated to hydrazide-bearing materials. This is particularly useful for immobilization of carbohydrates or glycoproteins.

Figure 4: Coupling procedure for TOYOPEARL AF-Epoxy-650M

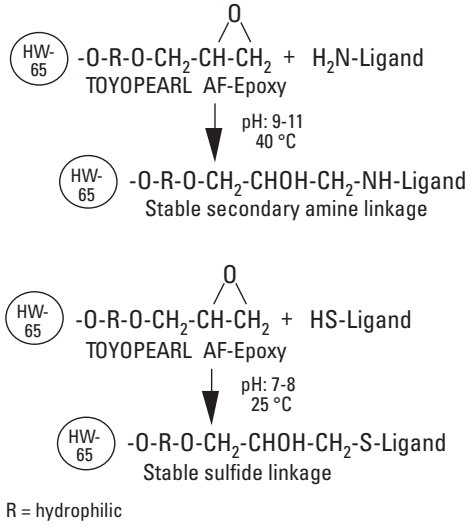


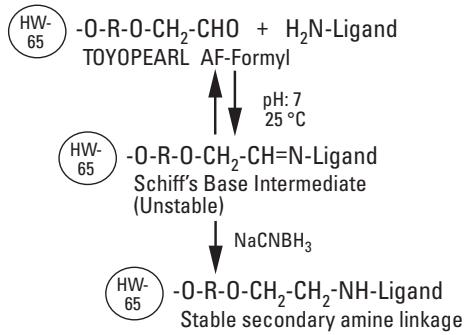
Table 1 Representative coupling densities for activated and reactive TOYOPEARL media

TOYOPEARL resin *Protein coupled (g/L resin)*	**AF-Tresyl-650M**	**AF-Formyl-650M**	**AF-Amino-650M**	**AF-Carboxy-650M**
soybean trypsin inhibitor	16	3.5	5.8	15
protein A	1.9	—	—	—
concanavalin A	13	—	—	—
α1-antitrypsin	12.3	—	—	—
α-chymotrypsin	12.5	—	—	—
myoglobin	12.4	—	—	—
ovalbumin	—	2.5	6.7	0.8
bovine serum albumin	12.4	14	19.2	3.3
human IgG	10.0	15	6.7	11.7
cytochrome	—	5.8	3.3	7.5
lysozyme	60	20	5.8	17.5
coupling agent	*not required*	*$NaCNBH_3$*	*$NaCNBH_3$ or carbodiimide*	*carbodiimide*
optimal pH	7.0 - 9.0	6.9 - 9.0	4.5 - 6.0	4.5 - 6.0

Reactive resins - require activation for ligand attachment

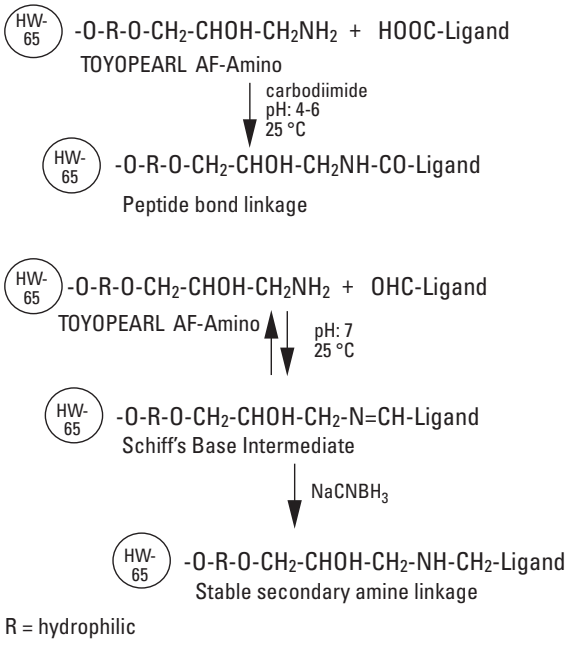
Ligands may be coupled to TOYOPEARL AF-Formyl-650M (aldehyde-bearing) resin under mild conditions exclusively using primary amines. The ligand is bound to the resin by a stable secondary amine linkage (Figure 5). A wide variety of industrial enzymes have been immobilized on aldehyde-bearing supports. Typically, these supports have been synthesized by industrial users by partial oxidation of polysaccharide supports (e.g. cellulose and agarose) or partial hydrolysis of polyacetals. In contrast, TOYOPEARL AF-Formyl-650M resin is a ready-to-use aldehyde support formulated from a dimensionally stable, macroporous matrix. Consistent aldehyde content and physical properties are ensured from batch to batch.

Figure 5: Coupling procedure for TOYOPEARL AF-Formyl-650M



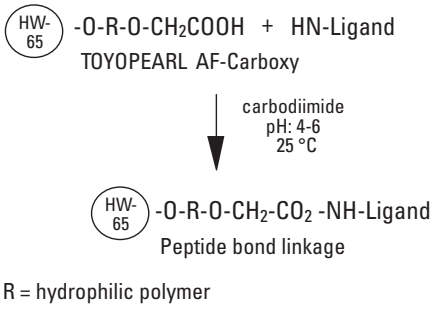
TOYOPEARL AF-Amino-650M resin may be used to couple ligands using their carboxyl groups through peptide bond formation or aldehyde groups by reductive amination as shown in Figure 6. Aldehyde groups may be present in a carbohydrate or glycoprotein ligand or may be introduced into the ligand by mild, periodate oxidation. The optimized functional group density of TOYOPEARL AF-Amino-650M (100 µmol/mL) is ideal for coupling of either proteins or low molecular weight ligands. For example, lactose was coupled by reductive alkylation to yield a ligand density of ca. 30 µmol/mL resin.

Figure 6: Coupling procedure for TOYOPEARL AF-Amino-650M



TOYOPEARL AF-Carboxy-650M resin provides another useful, though milder, approach for coupling to amino groups of proteins or low molecular weight ligands. The carbodiimide mediated coupling reaction produces an amide bond between ligand and support (Figure 7).

Figure 7: Coupling procedure for TOYOPEARL AF-Carboxy-650M



Resins with group specific ligands

TOYOPEARL AF-Chelate-650M resin is derivatized with iminodiacetic acid (IDA) at a concentration of ca. 20 µmol/mL. In typical applications, selected metal ions, most often Cu^{2+}, Ni^{2+}, Zn^{2+} and Co^{2+}, are bound to the support by stable chelation. The resultant metal ion-bearing resin binds to histidine and free cysteine containing sequences of a peptide or protein. Immobilized metal ion affinity chromatography (IMAC) has been used for purification of recombinant human growth factor, tissue plasminogen activator, glycophorins, and whole cells.

Functionalized with Cibachron Blue F3G-A, TOYOPEARL AF-Blue HC-650M resin has excellent capacity for proteins, particularly albumin (Figure 8). In addition, this high capacity resin has improved caustic stability, reduced dye ligand leakage, and superior pressure-flow characteristics relative to more traditional agarose materials (Figure 9).

Figure 8: Comparison of human serum albumin binding capacities at various pHs of TOYOPEARL AF-Blue HC-650M and Agarose (blue functionalized agarose) resins

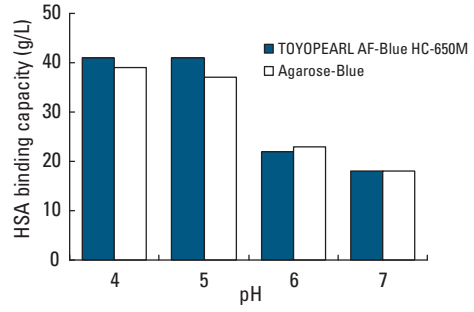
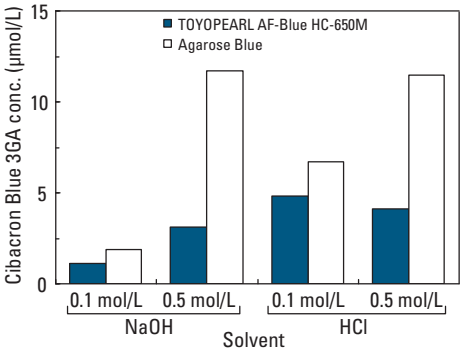


Figure 9: Comparative dye leakage study of TOYOPEARL AF-Blue HC-650M and Agarose Blue resins at 25 °C (after 24 hours)



TOYOPEARL AF-Red-650ML resins are functionalized with Procion Red HE-3B (also known as Reactive Red 120). This resin is useful for the purification of nucleotide-dependent enzymes, lipoproteins, plasminogen, peptides, hormones and cytotoxins. Both TOYOPEARL AF-Blue HC-650M and TOYOPEARL AF-Red-650ML resins are useful for the purification of nucleotide-dependent enzymes, albumin, cell growth factors, interferons, transferases, cyclases, and polymerases. Typical binding capacities are shown in Table 2.

Table 2: Representative binding capacities for TOYOPEARL dye-ligand affinity media

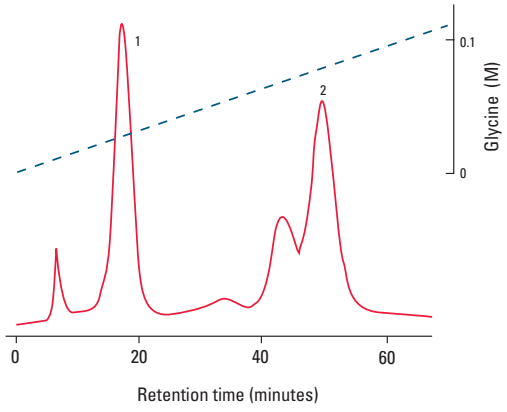
Protein (g/L)	TOYOPEARL AF-Blue HC-650M	TOYOPEARL AF-Red 650ML
hexokinase	3	—
bovine serum albumin	16	—
human serum albumin	18 ± 2.5	3.5 ± 1
lactate dehydrogenase	27	11

TOYOPEARL AF-Heparin HC-650M resin is a high capacity, affinity adsorbent with excellent chemical stability. The heparin ligand is a linear and highly sulfated glycosaminoglycan which has anti-coagulant properties. Due to its polyanionic nature, heparin interacts with a wide range of biomolecules including plasma components, lipoprotein lipase, collagenase, and DNA polymerase. Immobilized heparin is widely used as an adsorbent in affinity chromatography for the purification of biological substances.

Separation of Two Proteins

Metal ion affinity chromatography is often used for the purification of histidine-rich or histidine-tagged proteins. For example, in the separation of two proteins, zinc ions were immobilized to the resin and salt was used in the eluent to suppress the ionic interactions between the sample and the carboxyl groups of the AF-Chelate-650M resin (Figure 10). These conditions favor chelation of the proteins by the resin-bound metal ions over potential ion exchange interactions. Typical elution gradients use imidazole (1 mmol/L to 20 mmol/L), glycine (0 to 0.2 mol/L), or a pH gradient (8.0 to 4.0).

Figure 10: Immobilized metal ion affinity chromatography with TOYOPEARL AF-Chelate-650M

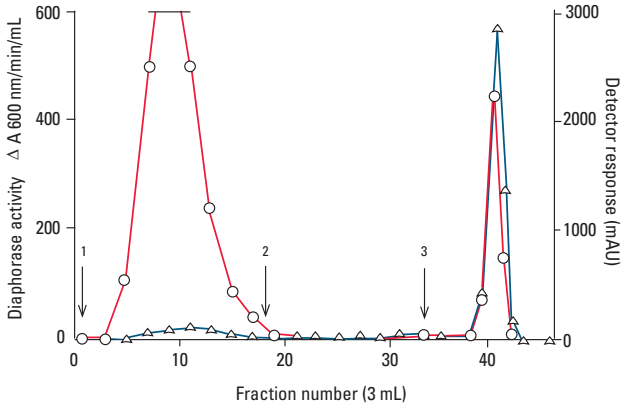


Resin:	**TOYOPEARL AF-Chelate-650M**
Column size:	8 mm ID × 7.5 cm
Metal ion:	Zn^{+2}
Mobile phase:	Buffer A: 20 mmol/L Tris-HCl buffer, 0.5 mol/L NaCl, pH 8.0 Buffer B: buffer A + 0.2 mol/L glycine
Gradient:	0 - 100 % B (120 min)
Flow rate:	60 cm/h (0.5 mL/min)
Detection:	UV @ 280 nm
Samples:	1. ribonuclease A. 250 µg 2. transferrin, 250 µg

Purification of Ferredoxin-NADP Reductase

Synechococcus ferredoxin (Fd) was coupled to TOYOPEARL AF-Tresyl using a 0.1 mol/L $NaHCO_3$, pH 8, coupling buffer. The resulting *Synechococcus* Fd-TOYOPEARL was used to purify ferredoxin-NADP reductase, as shown in Figure 11[1]. The TOYOPEAERL AF-Tresyl was preferred by the authors over agarose-based affinity resins due to the superior flow properties of the TOYOPEARL resin.

Figure 11: Affinity chromatography of spinach FNR on a Synechococcus Fd-TOYOPEARL column



Resin:	***Synechococcus* Fd-TOYOPEARL**
Column size:	22 mm ID × 10 cm
Mobile phase:	1. Load with crude spinach FNR in 20 mmol/L Tris-HCl, pH 7.5 2. Wash with 20 mmol/L Tris-HCl buffer, pH 7.5 3. Elute with 20 mmol/L Tris-HCl buffer, pH 7.5 with 0.25 mol/L NaCl
Flow rate:	16 cm/h (1 mL/min)
Detection:	UV @ 275 nm, specific activity
Sample:	spinach FNR

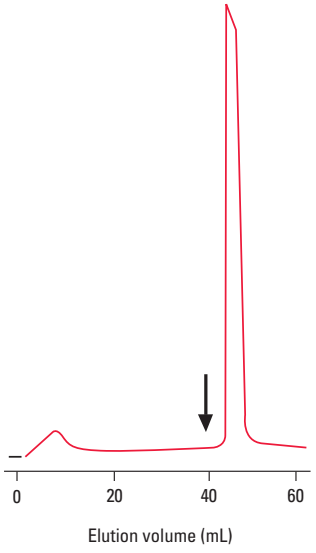
[1]Sakihama, N.; Nagai, K.; Ohmori, H.; Tomizawa, H.; Tsujita, M.; Shin, M. Immobilized ferredoxins for affinity chromatography of ferredoxin-dependent enzymes. *J. Chroma. A.* **1992**, *597*, 147-153.

Purification of Lectins

The high density of epoxy functionality is especially useful for generating specialized affinity supports with low molar mass ligands. For example, 150 mg N-acetylgalactosamine (GalNAc) was couple to 1.0 g of hydrated resin by reaction in 3 mL of 0.1 mol/L sodium hydroxide at 45 °C for 16 hours with gentle agitation[2]. The product was washed with distilled water, 1 mol/L sodium chloride, and distilled water. Residual epoxy groups were blocked by treatment with 1 mol/L ethanolamine (25 °C, 12 hours).

The TOYOPEARL AF-GalNAc-Epoxy resin was used to purify a lection from *Grifola frondosa* (GFL), an edible mushroom (Figure 12). A two-step affinity chromatography scheme yielded 3.2 mg of FGL with 86 % of the initial activity found in 2.34 g of crude protein from an ammonium sulfate precipitation.

Figure 12: Purification of lectins with specialized supports prepared from TOYOPEARL AF-Epoxy-650M



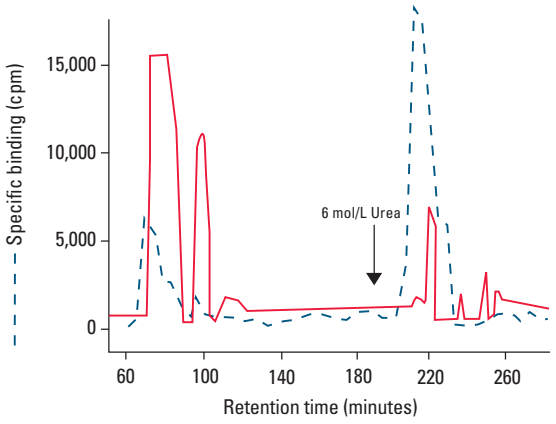
Resin:	**GalNAc-Epoxy TOYOPEARL**
Column size:	10 mm ID × 5 cm
Mobile phase:	Buffer A: 10 mmol/L phosphate-buffered saline, pH 7.4 Buffer B: buffer A + 0.1mol/L lactose
Gradient:	equilibrate/load/wash 100 % buffer A isocratic elution 100 % buffer B
Detection:	UV @ 275 nm
Sample:	4.0 mg impure *Grifola frondosa* lectin

[2]Kawagishi, H.; Nomura, A.; Mizuno, T.; Kimura, A.; Chiba. S. Isolation and characterization of a lectin from Grifola frondosa fruiting bodies. Biochimica et Biophysica Acta (BBA) - General Subjects. **1990**, *1034*, (3), 247-252.

Purification of GH Receptor Protein

As shown in Figure 13, growth hormone (GH) was coupled to TOYOPEARL AF-Formyl-650M, and then was used to purify GH receptor protein[3]. A size exclusion column (TSKgel G3000SW) was directly connected to the affinity column. This approach eliminated the urea that co-eluted with the GH receptor from the affinity column, and enabled high receptor activity as denaturation was minimized. This one-step procedure provided a 1,000-fold purification, yielding 50 mg of GH receptor.

Figure 13: Growth hormone immunoaffinity support prepared with TOYOPEARL AF-Formyl-650M



Resin:	**GH-Formyl TOYOPEARL**
Column size:	4.6 mm ID × 15 cm in series with TSKgel 3000SW, 7.6 mm ID × 60 cm
Mobile phase:	Buffer A: 50 mmol/L Tris-HCl buffer, 20 mmol/L NaCl, 10 mmol/L $MgCl_2$, 0.3 mmol/L phenylmethylsulfonyl fluoride GH receptor protein, 0.1 % Triton™ X-100, pH 7.4 Buffer B: buffer A + 6 mol/L urea
Gradient:	isocractic elution 100 % buffer B
Flow rate:	36 cm/h for 60 min, then 220 cm/h
Detection:	UV @ 280 nm, specific binding assay
Sample:	16 mg growth hormone (GH) receptor protein in 6 mL Triton X-100

[3]Yagi, S.; Izawa, K.; Nakagawa, T.; Tanaka, H.; Yoshitake, A.; Mohri, Z. Efficient high performance liquid chromatographic system for protein purification. *J. Chromatogr. A.* **1989**, *493*, (1), 27-33.

A selection of screening tools are available for TOYOPEARL Affinity resins. See the Process Development Products section of this Product Guide for details.

Ordering Information

TOYOPEARL Affinity resins:

Part #	Product description	Container size (mL)	Typical ligand density	Typical capacity (g/L)
TOYOPEARL Affinity Resins with Group Specific Ligands				
0019688	TOYOPEARL AF-Blue HC-650M	25	15 mmol/L	> 18 HSA
0019689	TOYOPEARL AF-Blue HC-650M	100		
0019690	TOYOPEARL AF-Blue HC-650M	1,000		
0019691	TOYOPEARL AF-Blue HC-650M	5,000		
0008651	TOYOPEARL AF-Red-650ML	25	7 mmol/L	2.5 - 4.5 HSA
0019801	TOYOPEARL AF-Red-650ML	100		
0042102	TOYOPEARL AF-Red-650ML	1,000		
0014475	TOYOPEARL AF-Chelate-650M	25	25 - 45 meq/L	≥ 60 lysozyme
0019800	TOYOPEARL AF-Chelate-650M	100		
0014907	TOYOPEARL AF-Chelate-650M	1,000		
0014908	TOYOPEARL AF-Chelate-650M	5,000		
0020030	TOYOPEARL AF-Heparin-HC-650M	10	5 g/L	≥ 5 AT III
0020031	TOYOPEARL AF-Heparin-HC-650M	100		
0020032	TOYOPEARL AF-Heparin-HC-650M	1,000		
0020033	TOYOPEARL AF-Heparin-HC-650M	5,000		
TOYOPEARL Reactive Affinity Resins				
0043411	TOYOPEARL AF-Amino-650M	10	70 - 130 meq/L	
0008002	TOYOPEARL AF-Amino-650M	25		
0008039	TOYOPEARL AF-Amino-650M	100		
0018074	TOYOPEARL AF-Amino-650M	1,000		
0018316	TOYOPEARL AF-Amino-650M	5,000		
0043412	TOYOPEARL AF-Carboxy-650M	10	80 - 120 meq/L	
0008006	TOYOPEARL AF-Carboxy-650M	25		
0008041	TOYOPEARL AF-Carboxy-650M	100		
0018827	TOYOPEARL AF-Carboxy-650M	1,000		
0018828	TOYOPEARL AF-Carboxy-650M	5,000		
0043413	TOYOPEARL AF-Formyl-650M	10	40 - 70 meq/L	
0008004	TOYOPEARL AF-Formyl-650M	25		
0008040	TOYOPEARL AF-Formyl-650M	100		
0017396	TOYOPEARL AF-Formyl-650M	1,000		
0017397	TOYOPEARL AF-Formyl-650M	5,000		

HSA = Human Serum Albumin

Part #	Product description	Container size (g)	Typical ligand density	Adsorption capacity (mg/g)
TOYOPEARL Activated Affinity Resins				
0043402	TOYOPEARL AF-Epoxy-650M*	5	600 - 1,000 µeq/g	> 60**
0008000	TOYOPEARL AF-Epoxy-650M*	10		
0008038	TOYOPEARL AF-Epoxy-650M*	100		
0018315	TOYOPEARL AF-Epoxy-650M*	1,000		
0014471	TOYOPEARL AF-Tresyl-650M*	5	80 mmol/L	≥ 60**
0014472	TOYOPEARL AF-Tresyl-650M*	100		
0014905	TOYOPEARL AF-Tresyl-650M*	200		
0014906	TOYOPEARL AF-Tresyl-650M*	1,000		
0018371	TOYOPEARL AF-Tresyl-650M*	5,000		

**Shipped dry. 1 g yields approximately 3.5 mL of hydrated resin*
***Measured as amount of test protein coupled per gram of dry gel.*

TOYOPEARL MX-Trp-650M

The role of Mixed-Mode Chromatography in Process Purification

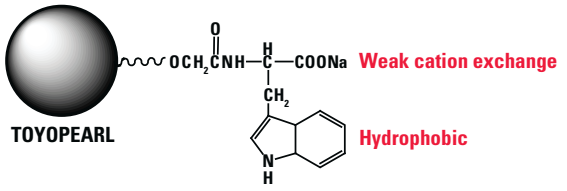
Multimodal or mixed-mode chromatography resins are based on media that have been functionalized with ligands inherently capable of several different types of interaction: ion exchange, affinity, size exclusion and hydrophobic. The ability to merge and take advantage of these modes of protein separations can enhance overall selectivity in a purification process. This enhanced selectivity can be used to remove process impurities in a single column step that would otherwise require multiple processing steps to remove. Mixed-mode resins are in effect an amalgamation of complementary approaches to chromatographic separation on a single platform.

Unlike monomodal chromatographic methods where molecules are separated based on a single characteristic (activity, charge or hydrophobicity), with mixed-mode chromatography and mixed-mode ligands, there is no known single specific interaction between the ligand and the molecule of interest. As such, screening mixed-mode resins becomes an exploration for sites on the target molecule that will deliver suitable selectivity and capacity. It is recommended that chromatographers screen for pH and conductivity as well as loading conditions when optimizing a purification process that incorporates mixed-mode resins. Protein-ligand interactions are not independent of one another on mixed-mode resins. For example, when using a mixed-mode resin having both hydrophobic interaction and ion exchange components, increasing conductivity will interrupt ionic bonds while at the same time enhancing any hydrophobic interactions. Because multiple dependent and independent variables are involved in using mixed-mode chromatography, the use of Design-of-Experiments (DoE) is recommended to characterize and optimize chromatographic conditions.

TOYOPEARL Mixed-Mode Chromatography Resin

TOYOPEARL MX-Trp-650M resin is a functionalized version of the TOYOPEARL HW size exclusion resin and is therefore based on a hydroxylated polymethacrylic polymer bead. Tosoh Bioscience offers one mixed-mode ligand, the amino acid tryptophan, which has both indole hydrophobic and weak carboxyl cationic functional groups (Figure 1).

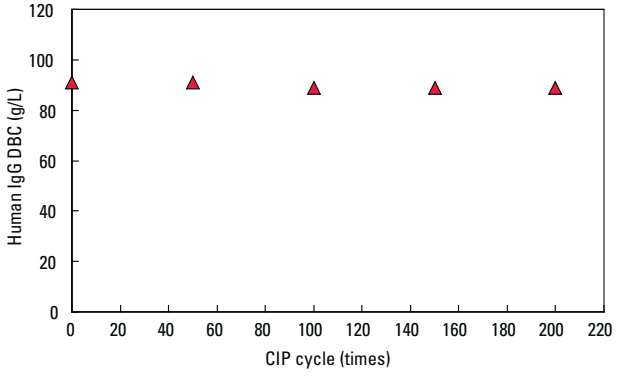
Figure 1: TOYOPEARL MX-Trp-650M structure



Product name: TOYOPEARL MX-Trp-650M
Particle size: 50-100 µm

The semi-rigid polymeric backbone of TOYOPEARL MX-Trp-650M permits high flow rates for maximum throughput and productivity. This mixed-mode resin may be operated at pressures up to 0.3 MPa and is chemically stable from pH 3-13. This allows a constant packing volume over a wide range of salt concentrations and cleaning in place (CIP) with acid or base. As shown in Figure 2, TOYOPEARL MX-Trp-650M has excellent stability to 0.5 mol/L NaOH and can be run for many CIP cycles without decreasing dynamic binding capacity (DBC).

Figure 2: Stability in 0.5 mol/L NaOH



Resin: **TOYOPEARL MX-Trp-650M**

Alkaline cleaning (CIP) conditions
- 3CV: 0.5 mol/L NaOH,
- 5CV: 0.1 mol/L Tris-HCl buffer, pH 8.5 + 0.3 mol/L NaCl
- Flow rate: 212 cm/h (1.0 mL/min)

DBC Measurement
- Column size: 6 mm ID × 4 cm
- Binding buffer: 0.05 mol/L acetate buffer, pH 4.7 + 0.1 mol/L NaCl
- Flow rate: 212 cm/h (1 mL/min)
- Detection: UV @ 280 nm
- Sample: polyclonal human IgG
- Sample Load: 1 mg/mL

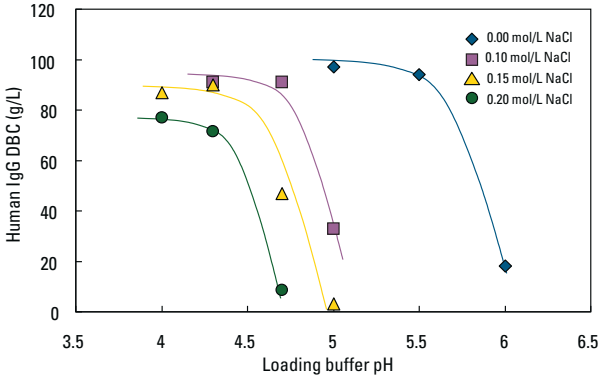
Table 1: Properties of TOYOPEARL MX-Trp-650M resin

TOYOPEARL resin	Functionality	Base bead	Pore size	Bead diameter	Ligand type	Ligand pKa ($-CO_2H$)*	DBC (g/L)	Pressure rating
MX-Trp-650M	cationic/HIC	HW-65	100 nm	75 µm	HIC/ weak cation	2.38	90 - 100	0.3 MPa

**Ligand pKa value is the pKa of the α-carboxyl group on the amino acid itself.*

TOYOPEARL MX-Trp-650M is a high capacity mixed-mode resin used for the purification of monoclonal antibodies and other proteins. The multimodal resin maintains DBC at elevated feedstock or buffer conductivities (Figure 3). Table 2 shows the DBC of TOYOPEARL MX-Trp-650M at two feedstock conductivities: 12 mS/cm and 17 mS/cm. For comparison purposes, data for an agarose based resin is also shown. For the 12 mS/cm and 17 mS/cm measurements, the TOYOPEARL MX-Trp-650M resin shows almost 7× higher and 4× higher DBC, respectively, than the agarose based resin. Superior product recovery over the agarose based resin is also demonstrated in Table 3.

Figure 3: Effect of buffer pH and salt on DBC for TOYOPEARL MX-Trp-650M



Resin: **TOYOPEARL MX-Trp-650M**
Column: 6 mm ID × 4 cm
Mobile phase: Buffer A: 0.05 mol/L acetate buffer, pH 4.0 - 6.0 + 0 - 0.2 mol/L NaCl
Buffer B: 0.1 mol/L Tris-HCl buffer, pH 8.5 + 0.3 mol/L NaCl
Flow rate: 212 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Sample: human polyclonal IgG (1 g/L)

Dynamic binding capacity (DBC) calculated from 10 % height of breakthrough curve

Table 2: Dynamic binding capacities at high conductivities

Resin	Particle size(µm)	Ion exchange capacity (meq)	DBC (g/L)	Recovery %
TOYOPEARL MX-Trp-650M (12 mS/cm)	50 - 100	0.12	95	97
TOYOPEARL MX-Trp-650M (17 mS/cm)	50 - 100	0.12	48	96
Brand M (Agarose 12 mS/cm)	75 (median)	0.24	14	86
Brand M (Agarose 17 mS/cm)	75 (median)	0.24	11	85

Resins: **TOYOPEARL MX-Trp-650M**
Brand M
Column size: 6 mm ID × 4 cm
Mobile phase: Buffer (12 mS/cm): 0.05 mol/L acetate buffer, pH 4.3, 4.7, 5.0 + 0.10 mol/L NaCl
Buffer (17 mS/cm): 0.05 mol/L acetate buffer, pH 4.3, 4.7, 5.0 + 0.15 mol/L NaCl
Flow rate: 212 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Sample: human polyclonal IgG (1 g/L)

Dynamic binding capacity (DBC) calculated from 10 % height of breakthrough curve.

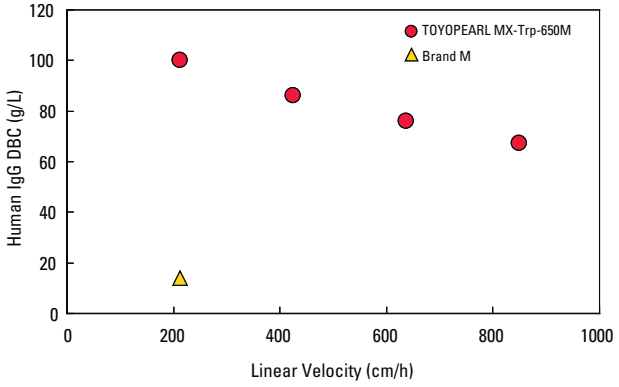
Table 3: Recovery comparison at conductivities of 12 and 17 mS/cm

Resin	IgG DBC 12 mS/cm	Recovery 12 mS/cm	IgG DBC 17 mS/cm	Recovery 17 mS/cm
TOYOPEARL MX-Trp-650M	95	97 %	48	96 %
Capto MMC	14	86 %	11	85 %

Resins: **TOYOPEARL MX-Trp-650M**
Capto MMC
Column size: 6 mm ID × 4 cm
Mobile phase: Buffer (12 mS/cm): 0.05 mol/L acetate buffer, pH 4.7 + 0.1 mol/L NaCl
Buffer (17 mS/cm): 0.05 mol/L acetate buffer, pH 4.7 + 0.15 mol/L NaCl
Flow rate: 212 cm/h (1.0 mL/min)
Detection: UV @ 280 nm
Sample: polyclonal IgG

The mass transfer properties of a resin influence the economics of the loading and elution stages of a capture step and the degree of resolution for intermediate purification. Good mass transfer kinetics enables the resin to maintain its DBC at increased linear velocities (Figure 4). In keeping with the exceptional target binding and eluting properties of TOYOPEARL GigaCap ion exchange resins, TOYOPEARL MX-Trp-650M also shows a narrow elution peak width to complement its higher capacity (Figure 5). The mass transfer properties also contribute to minimal peak broadening. Figure 6 shows the excellent peak shape for TOYOPEARL MX-Trp-650M and the much broader tailing associated with the Brand M agarose material.

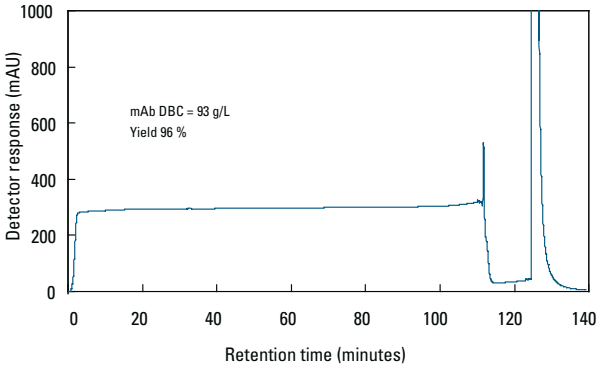
Figure 4: DBC at higher linear velocities



Resins:	**TOYOPEARL MX-Trp-650M**
	Brand M
Column size:	0.6 mm ID × 4.0 cm
Mobile phase:	Buffer A: 0.05 mol/L sodium acetate buffer, pH 4.7 + 0.1 mol/L sodium chloride
	Buffer B: 0.1 mol/L Tris-HCl buffer, pH 8.5 + 0.3 mol/L sodium chloride
Flow rates:	212, 425, 637, 849 cm/hr (1, 2, 3, 4 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	polyclonal human IgG (1 g/L)
Sample load:	1 mg/mL

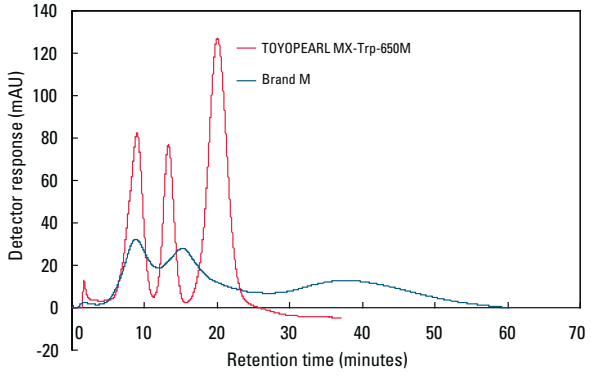
Dynamic binding capacities (DBC) were determined at 10 % breakthrough

Figure 5: Narrow elution peak widths



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 0.05 mol/L acetate buffer, pH 4.7 + 0.1 mol/L NaCl (12 mS/cm)
	Buffer B: 0.1 mol/L Tris-HCl buffer, pH 8.5 + 0.3 mol/L NaCl
Flow rate:	A: 212 cm/h (1.0 mL/min)
	B: 424 cm/h (2.0 mL/min) started at 124 min
Detection:	UV @ 280 nm
Sample:	CHO cell culture media, monoclonal antibody (1 mg/mL) diluted with buffer A

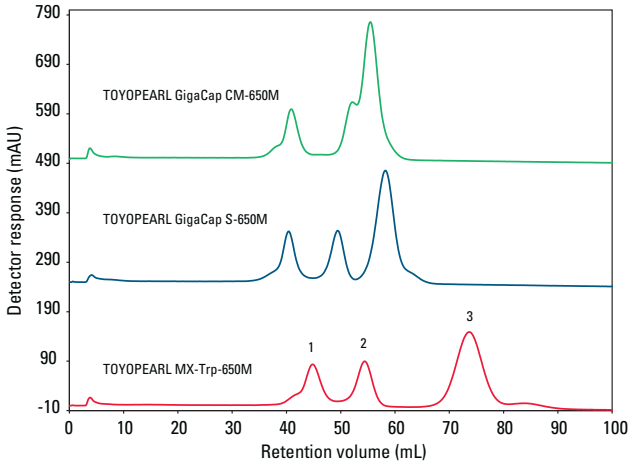
Figure 6: Good resolution for intermediate purification



Resins:	**TOYOPEARL MX-Trp-650M, Brand M**
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	Buffer A: 20 mmol/L phosphate buffer, pH 7.0
	Buffer B: 20 mmol/L phosphate buffer + 1.0 mol/L NaCl, pH 7.0
Gradient:	30 min. linear gradient from buffer A to buffer B
Flow rate:	136 cm/h (1.0 mL/min)
Detection:	UV @ 280 nm
Samples:	trypsinogen (6.6 g/L)
	cytochrome C (3.6 g/L)
	lysozyme (6.6 g/L)
Load volume:	25 µL

Selectivity of TOYOPEARL MX-Trp-650M, when compared to a traditional weak cation exchange (TOYOPEARL GigaCap CM-650M) and a traditional strong cation exchange (TOYOPEARL GigaCap S-650M) resin, is noticeably different. A three protein mixture (trypsinogen, cytochrome C, and lysozyme) was loaded onto each resin in 20 mmol/L sodium phosphate buffer (pH 7.0) and eluted with a linear salt gradient (Figure 7). Resolution between the peaks was measured and recorded for comparison (Table 4). Further selectivity comparisons were done at decreasing pH levels for all three resins with the same protein mixture at pH 6.0 (20 mmol/L sodium acetate) and pH 5.0 (20 mmol/L sodium citrate) and were compared to the initial screening at pH 7.0 (Figures 8-10). Resolution between the peaks was likewise measured and recorded for comparison (Tables 5-7).

Figure 7: Initial selectivity screening

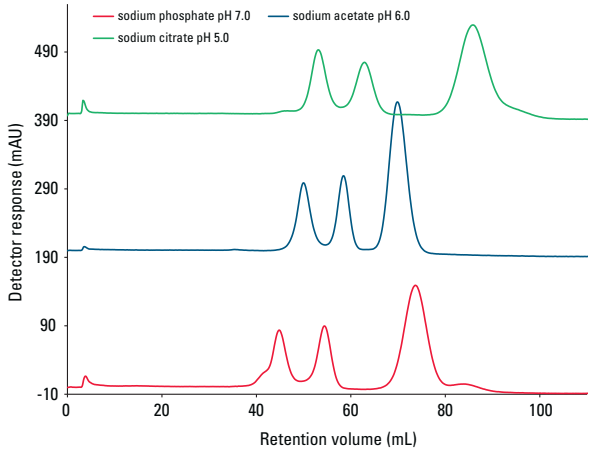


Resin:	**As Indicated**
Column size:	6.6 mm ID × 15.5 ± 1.0 cm
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate buffer, pH 7.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0 % B - 100 % B
Flow rate:	200 cm/h (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	1. trypsinogen (6.6 g/L) 2. cytochrome C (3.6 g/L) 3. lysozyme (6.6 g/L)
Sample load:	5 % CV (4.02 - 4.60 mg total protein)

Table 4: Initial selectivity screening peak resolutions

	Peak resolution	
Resin	**trypsinogen/ cytochrome C**	**cytochrome C/ lysozyme**
TOYOPEARL MX-Trp-650M	0.81	1.50
TOYOPEARL GigaCap S-650M	0.94	0.82
TOYOPEARL GigaCap CM-650M	1.40	0.43

Figure 8: TOYOPEARL MX-Trp-650M pH scouting

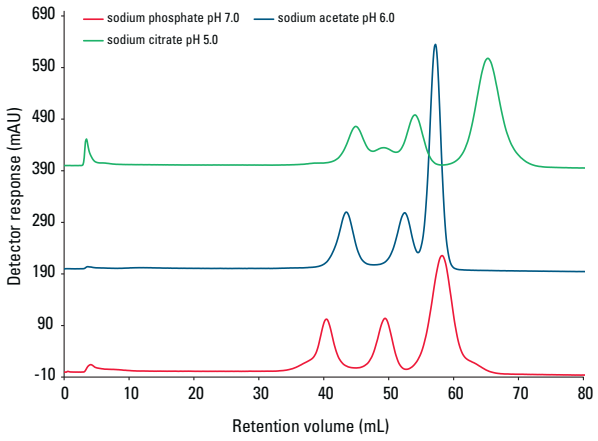


Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate buffer, pH 7.0 Buffer A: 20 mmol/L sodium acetate buffer, pH 6.0 Buffer A: 20 mmol/L citrate, pH 5.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0 % B - 100 % B
Flow rate:	200 cm/h (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	1. trypsinogen (6.6 g/L) 2. cytochrome C (3.6 g/L) 3. lysozyme (6.6 g/L)
Sample load:	5 % CV (4.45 mg total protein)

Table 5: TOYOPEARL MX-Trp-650M pH scouting peak resolutions

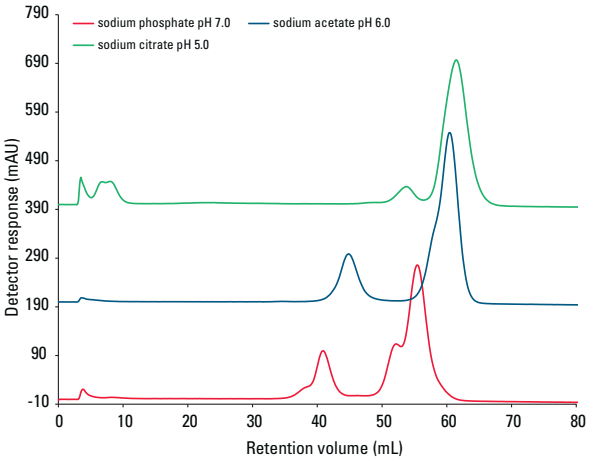
	Trypsinogen retention (mL)	Cytochrome C retention (mL)	Trypsinogen/ cytochrome C resolution (Rs)	Lysozyme retention (mL)	Cytochrome C/ lysozyme resolution (Rs)
Phosphate pH 7.0	44.88	54.36	0.81	73.63	1.50
Acetate pH 6.0	50.01	58.45	0.89	69.87	1.04
Citrate pH 5.0	53.08	62.94	1.07	85.97	1.57

Figure 9: TOYOPEARL GigaCap S-650M pH scouting



Resin:	**TOYOPEARL GigaCap S-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate buffer, pH 7.0 Buffer A: 20 mmol/L sodium acetate buffer, pH 6.0 Buffer A: 20 mmol/L citrate buffer, pH 5.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100 % B
Flow rate:	200 cm/h (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	1. trypsinogen (6.6 g/L) 2. cytochrome C (3.6 g/L) 3. lysozyme (6.6 g/L)
Sample load:	5 % CV (4.31 mg total protein)

Figure 10: TOYOPEARL GigaCap CM-650M pH scouting



Resin:	**TOYOPEARL GigaCap CM-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate buffer, pH 7.0 Buffer A: 20 mmol/L sodium acetate buffer, pH 6.0 Buffer A: 20 mmol/L citrate buffer, pH 5.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0 % B - 100 % B
Flow rate:	200 cm/h (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	1. trypsinogen (6.6 g/L) 2. cytochrome C (3.6 g/L) 3. lysozyme (6.6 g/L)
Sample load:	5 % CV (4.31 mg total protein)

Table 6: TOYOPEARL GigaCap S-650M pH scouting peak resolutions

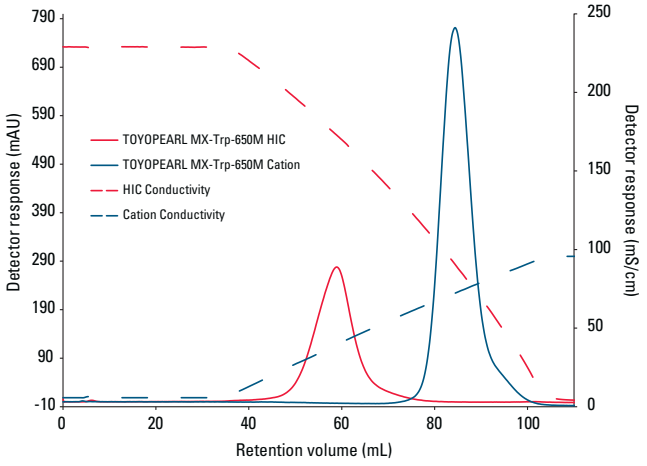
	Trypsinogen retention (mL)	Cytochrome C retention (mL)	Trypsinogen/ cytochrome C resolution (Rs)	Lysozyme retention (mL)	Cytochrome C/ lysozyme resolution (Rs)
Phosphate pH 7.0	40.38	49.46	0.94	58.27	0.82
Acetate pH 6.0	43.44	52.46	1.16	57.20	0.75
Citrate pH 5.0	44.96	54.05	1.23	65.29	1.00

Table 7: TOYOPEARL GigaCap CM-650M pH scouting peak resolutions

	Trypsinogen retention (mL)	Cytochrome C retention (mL)	Trypsinogen/ cytochrome C resolution (Rs)	Lysozyme retention (mL)	Cytochrome C/ lysozyme resolution (Rs)
Phosphate pH 7.0	40.89	52.20	1.40	55.45	0.43
Acetate pH 6.0	44.81	60.46	1.18	60.46	0
Citrate pH 5.0	53.71	61.46	0.84	61.46	0

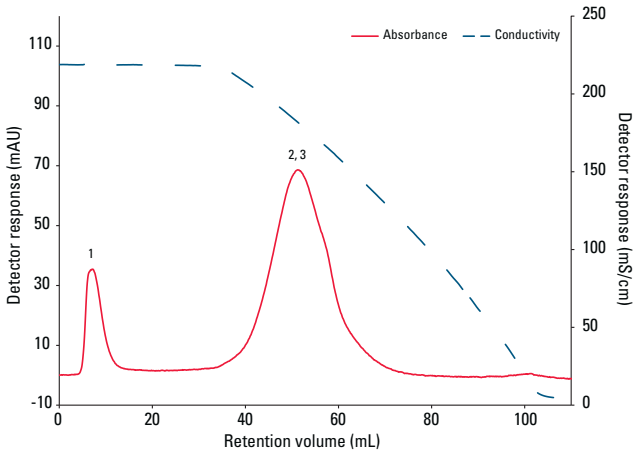
To examine the role the hydrophobic region of the tryptophan ligand can play in protein separations on TOYOPEARL MX-Trp-650M, the resin was tested to determine if it was possible to be used solely in HIC mode by loading lysozyme onto the column in 10 mmol/L sodium citrate, 1.8 mol/L ammonium sulfate, pH 5.0. The bound lysozyme was eluted with a decreasing linear gradient of 10 mmol/L sodium citrate, pH 5.0 (Figure 11). Comparison of resin selectivity in HIC mode and weak cation mode was done using a three protein mix (ribonuclease A, α-chymotrypsinogen, and lysozyme) at pH 5.0 with sodium citrate as the mobile phase buffering salt (Figure 12 and 13). Further selectivity experiments with TOYOPEARL MX-Trp-650M can be found in *AN44: TOYOPEARL MX-Trp-650M Salt Selectivity and Tolerance.*

Figure 11: TOYOPEARL MX-Trp-650M HIC functionality with cation comparison



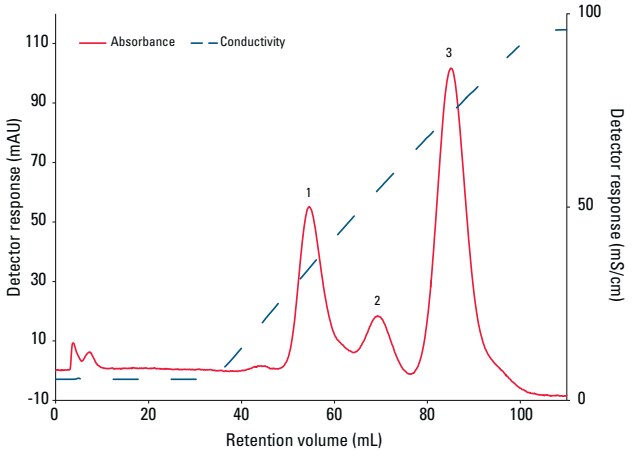
Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A (HIC): 10 mmol/L sodium citrate buffer, 1.8 mol/L ammonium sulfate buffer, pH 5.0 Buffer B (HIC): 10 mmol/L sodium citrate buffer, pH 5.0 Buffer A (cation): 20 mmol/L sodium citrate buffer, pH 5.0 Buffer B (cation): buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0 % B - 100 % B
Flow rate:	200 cm/h (1.14 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Temperature:	ambient
Sample:	lysozyme (cation – 10 mg/mL; HIC – 4 g/L)
Sample load:	5 % CV (1.06 and 2.65 mg total protein)

Figure 12: TOYOPEARL MX-Trp-650M HIC selectivity



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A (HIC): 10 mmol/L sodium citrate buffer, 1.8 mol/L ammonium sulfate buffer, pH 5.0 Buffer B (HIC): 10 mmol/L sodium citrate buffer, pH 5.0
Gradient:	60 minutes 0 % B - 100 % B
Flow rate:	200 cm/h (1.14 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Temperature:	ambient
Samples:	1. ribonuclease A (4.0 g/L) 2. α-chymotrypsinogen (5.0 g/L) 3. lysozyme (6.0 g/L)
Sample load:	5 % CV (3.98 mg total protein)

Figure 13: TOYOPEARL MX-Trp-650M cation selectivity



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A (cation): 20 mmol/L sodium citrate buffer, pH 5.0 Buffer B (cation): buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0 % B - 100 % B
Flow rate:	200 cm/h (1.14 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Temperature:	ambient
Samples:	1. ribonuclease A (4.0 g/L) 2. α-chymotrypsinogen (5.0 g/L) 3. lysozyme (6.0 g/L)
Sample load:	5 % CV (3.98 mg total protein)

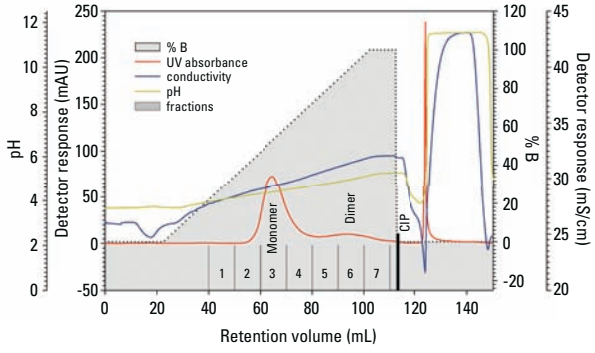
Parameters to Consider when Using TOYOPEARL MX-Trp-650M

Coordinating the hydrophobicity and charge of the therapeutic target to TOYOPEARL MX-Trp-650M is critical for the best overall purification performance. Operating at the extremes of hydrophobicity or charge for a given protein can result in drastically reduced performance of the resin or in some cases, a loss of biological activity. An optimum mixed-mode process step will balance high dynamic binding capacity, adequate selectivity, good mass recovery, and retention of biological activity. Execution of a DoE protocol during the screening process will enable developers to optimize protein separations by fine tuning mobile phase pH, conductivity and product load parameters.

Separation of Aggregates from mAbs

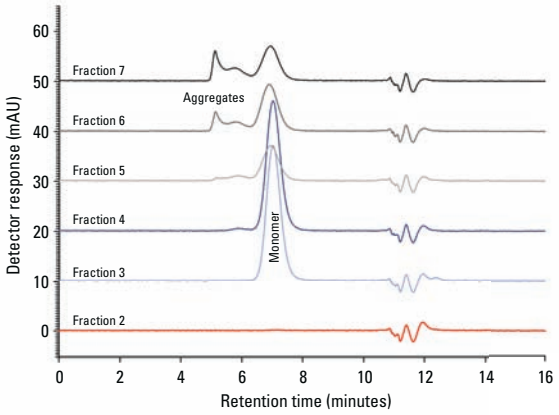
TOYOPEARL MX-Trp-650M successfully removes mAb aggregate from monomer using a narrow combination gradient of pH and conductivity (the pH and salt concentration range from pH 4.0 to 6.0 and 0.2 mol/L NaCl to 0.4 mol/L NaCl) respectively (Figure 14). The aggregate content in the monomer pool is below 1 %, as shown in SEC chromatograms of the collected fractions analyzed in Figure 15. From these results it can be seen that TOYOPEARL MX-Trp-650M can be utilized as a highly efficient tool for aggregate removal of mAbs, as it offers capacities comparable to IEX, high recovery, and excellent selectivity.

Figure 14: Separation of mAb monomers and aggregates



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 2.0 cm
Mobile phase:	Buffer A: 0.1 mol/L acetate buffer + 0.2 mol/L NaCl, pH 4.3 Buffer B: 0.1 mol/L acetate buffer + 0.4 mol/L NaCl, pH 5.6
Flow rate:	150 cm/h (0.86 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Sample:	10 mg mAb + mAb aggregates
Sample load:	1 g/L

Figure 15: SEC chromatograms of the collected fractions



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 2.0 cm
Mobile phase:	Buffer A: 0.1 mol/L acetate buffer + 0.2 mol/L NaCl, pH 4.3 Buffer B: 0.1 mol/L acetate buffer + 0.4 mol/L NaCl, pH 5.6
Flow rate:	150 cm/h (0.86 mL/min)
Detection:	UV @ 280 nm
Sample:	10 mg mAb + mAb aggregates
Sample load:	1 g/L

A selection of screening tools are available for TOYOPEARL Mixed-Mode resin. See the Process Development Products section of this Product Guide for details.

Ordering Information

TOYOPEARL Mixed-mode resin:

Part #	Product description	Container size (mL)	Bead diameter (µm)	IgG capacity (g/L)
0022817	TOYOPEARL MX-Trp-650M	25	50 - 100	≥75
0022818	TOYOPEARL MX-Trp-650M	100		
0022819	TOYOPEARL MX-Trp-650M	1,000		
0022820	TOYOPEARL MX-Trp-650M	5,000		


TOSOH
TOYOPEARL®
MX-Trp-650M
FOR IN VITRO USE ONLY / FOR PACKED-BED USE ONLY
Net Vol. 100 mL
Part No. 0022818
Lot No.
Made in Japan
TOSOH
TOYOPEARL®
MX-Trp-650M
FOR IN VITRO USE ONLY / FOR PACKED-BED USE ONLY
Net Vol. 100 mL
Part No. 0022818
Lot No.
Made in Japan
TOSOH

ToyoScreen process development columns

ToyoScreen RoboColumns®

TOYOPEARL and TSKgel LabPak media

Resin Seeker 96-well plate kits

TOYOPEARL and TSKgel bulk media (<1 L)

TOYOPEARL protein A ELISA kits

About: ToyoScreen Process Development Columns

The first part of the development process is the screening and selection of a resin. Due to the diversity in available ligand chemistries and base matrices offered by different vendors (e.g., agarose, methacrylate, styrene/divinylbenzene etc.) it is prudent at the development stage to screen as many resins as possible. A thorough evaluation is a necessity as each target molecule has very different physical and chromatographic properties. Very often a resin that worked in the past for a similar molecule will not work as effectively for the new target molecule.

Historically, resin screening was accomplished by manually packing various bulk resins into small columns requiring a significant investment in time and cost. In order to improve the efficiency of resin screening experiments, pre-packed ToyoScreen columns containing the most popular TOYOPEARL resins now provide a low cost and convenient alternative for the evaluation of TOYOPEARL ligand chemistries.

The 1 mL and 5 mL ToyoScreen columns are available in single or mixed chemistry packs and offer seamless integration with any platform (HPLC, FPLC, and appropriate AKTA® systems).

Ordering Information - ToyoScreen process development columns

Part #	Description	Matrix	Container size
Ion Exchange			
0021366	ToyoScreen CM-650M, 1 mL	polymer	1 mL × 6 ea
0021367	ToyoScreen CM-650M, 5 mL		5 mL × 6 ea
0021360	ToyoScreen DEAE-650M, 1 mL		1 mL × 6 ea
0021361	ToyoScreen DEAE-650M, 5 mL		5 mL × 6 ea
0022872	ToyoScreen GigaCap DEAE-650M, 1 mL		1 mL × 6 ea
0022873	ToyoScreen GigaCap DEAE-650M, 5 mL		5 mL × 6 ea
0021859	ToyoScreen GigaCap Q-650M, 1 mL		1 mL × 6 ea
0021860	ToyoScreen GigaCap Q-650M, 5 mL		5 mL × 6 ea
0021868	ToyoScreen GigaCap S-650M, 1 mL		1 mL × 6 ea
0021869	ToyoScreen GigaCap S-650M, 5 mL		5 mL × 6 ea
0021951	ToyoScreen GigaCap CM-650M, 1 mL		1 mL × 6 ea
0021952	ToyoScreen GigaCap CM-650M, 5 mL		5 mL × 6 ea
0021870	ToyoScreen MegaCap II SP-550EC, 1 mL		1 mL × 6 ea
0021871	ToyoScreen MegaCap II SP-550EC, 5 mL		5 mL × 6 ea
0021362	ToyoScreen SuperQ-650M, 1 mL		1 mL × 6 ea
0021363	ToyoScreen SuperQ-650M, 5 mL		5 mL × 6 ea
0021992	ToyoScreen Q-600C AR, 1 mL		1 mL × 6 ea
0021993	ToyoScreen Q-600C AR, 5 mL		5 mL × 6 ea
0021364	ToyoScreen QAE-550C, 1 mL		1 mL × 6 ea
0021365	ToyoScreen QAE-550C, 5 mL		5 mL × 6 ea
0021370	ToyoScreen SP-550C, 1 mL		1 mL × 6 ea
0021371	ToyoScreen SP-550C, 5 mL		5 mL × 6 ea
0021368	ToyoScreen SP-650M, 1 mL		1 mL × 6 ea
0021369	ToyoScreen SP-650M, 5 mL		5 mL × 6 ea
Hydrophobic Interaction			
0021494	ToyoScreen Butyl-600M, 1 mL	polymer	1 mL × 6 ea
0021495	ToyoScreen Butyl-600M, 5 mL		5 mL × 6 ea
0021376	ToyoScreen Butyl-650M, 1 mL		1 mL × 6 ea
0021377	ToyoScreen Butyl-650M, 5 mL		5 mL × 6 ea
0021372	ToyoScreen Ether-650M, 1 mL		1 mL × 6 ea
0021373	ToyoScreen Ether-650M, 5 mL		5 mL × 6 ea
0021378	ToyoScreen Hexyl-650C, 1 mL		1 mL × 6 ea
0021379	ToyoScreen Hexyl-650C, 5 mL		5 mL × 6 ea

Part #	Description	Matrix	Container size
0021892	ToyoScreen Phenyl-600M, 1 mL	polymer	1 mL × 6 ea
0021893	ToyoScreen Phenyl-600M, 5 mL		5 mL × 6 ea
0021374	ToyoScreen Phenyl-650M, 1 mL		1 mL × 6 ea
0021375	ToyoScreen Phenyl-650M, 5 mL		5 mL × 6 ea
0021380	ToyoScreen PPG-600M, 1 mL		1 mL × 6 ea
0021381	ToyoScreen PPG-600M, 5 mL		5 mL × 6 ea
0021382	ToyoScreen SuperButyl-550C, 1 mL		1 mL × 6 ea
0021383	ToyoScreen SuperButyl-550C, 5 mL		5 mL × 6 ea
Mixed-Mode			
0022824	ToyoScreen MX-Trp-650M, 1 mL	polymer	1 mL × 6 ea
0022825	ToyoScreen MX-Trp-650M, 5 mL		5 mL × 6 ea
Affinity			
0021386	ToyoScreen AF-Blue HC-650M, 1 mL	polymer	1 mL × 6 ea
0021387	ToyoScreen AF-Blue HC-650M, 5 mL		5 mL × 6 ea
0021384	ToyoScreen AF-Chelate-650M, 1 mL		1 mL × 6 ea
0021385	ToyoScreen AF-Chelate-650M, 5 mL		5 mL × 6 ea
0021390	ToyoScreen AF-Heparin HC-650M, 1 mL		1 mL × 6 ea
0021391	ToyoScreen AF-Heparin HC-650M, 5 mL		5 mL × 6 ea
0021388	ToyoScreen AF-Red-650M, 1 mL		1 mL × 6 ea
0021389	ToyoScreen AF-Red-650M, 5 mL		5 mL × 6 ea
Protein A			
0022809	ToyoScreen AF-rProtein A-650F, 1 mL	polymer	1 mL × 5 ea
0022810	ToyoScreen AF-rProtein A-650F, 5 mL		5 mL × 1 ea
0022811	ToyoScreen AF-rProtein A-650F, 5 mL		5 mL × 5 ea
0023430	ToyoScreen AF-rProtein A HC-650F, 1 mL		1 mL × 5 ea
0023431	ToyoScreen AF-rProtein A HC-650F, 5 mL		5 mL × 1 ea
0023432	ToyoScreen AF-rProtein A HC-650F, 5 mL		5 mL × 5 ea
Anion Mix Pack (DEAE-650M, SuperQ-650M, QAE-550C, GigaCap Q-650M, Q-600C AR)			
0021392	ToyoScreen IEC Anion Mix Pack, 1 mL	polymer	1 mL × 5 grades × 1 each
0021393	ToyoScreen IEC Anion Mix Pack, 1 mL		5 mL × 5 grades × 1 each
Cation Mix Pack (CM-650M, SP-650M, SP-550C, GigaCap CM-650M, GigaCap S-650M)			
0021394	ToyoScreen IEC Cation Mix Pack, 1 mL	polymer	1 mL × 5 grades × 1 each
0021395	ToyoScreen IEC Cation Mix Pack, 5 mL		5 mL × 5 grades × 1 each
IEX Mix Pack (GigaCap Q-650M, SuperQ-650M, Q-600C AR, GigaCap CM-650M, GigaCap S650M, SP-550C)			
0021396	ToyoScreen IEC Mix Pack, 1 mL	polymer	1 mL × 5 grades × 1 each
0021397	ToyoScreen IEC Mix Pack, 5 mL		5 mL × 5 grades × 1 each
HIC Mix Pack (PPG-600M, Phenyl-600M, Phenyl-650M, Butyl-600M, Butyl-650M, Hexyl-650C)			
0021398	ToyoScreen HIC Mix Pack, 1 mL	polymer	1 mL × 5 grades × 1 each
0021399	ToyoScreen HIC Mix Pack, 5 mL		5 mL × 5 grades × 1 each
ToyoScreen Accessories			
0021400	ToyoScreen Column Holder		
0042194	ToyoScreen Holder with fittings		Incl. 1 × 21400, 2 × 42196, 1 × 42195
0042195	Column Coupler, 10-32, 0.03"ID SS Tubing		
0042196	Adapter, M6 interior to 10-32 exterior, PEEK		
0042197	Adapter, 1/4-28 interior to 10-32 exterior, PEEK		

About: ToyoScreen RoboColumns

ToyoScreen RoboColumns are miniaturized chromatographic columns pre-packed with TOYOPEARL ion exchange, mixed-mode, hydrophobic interaction or affinity media. Available in different volumes, they are designed to operate with a robotic liquid handling system, such as the Freedom EVO® from TECAN.

This approach allows automated high throughput, small-scale biochromatographic separations of protein samples by running up to eight individual columns simultaneously.

ToyoScreen RoboColumns are packed with TOYOPEARL resins to our specifications by Atoll GmbH and are supplied in strips of 8 columns.

ToyoScreen RoboColumns can be used in a wide range of applications, including individual and parallel resin screening, optimization of separation conditions, scale-down experiments, as well as high throughput sample preparation. Binding and elution conditions, washing parameters, etc. can be investigated to explore the design space of the particular molecule's purification process.

Ordering Information - ToyoScreen RoboColumns

Part #	Packed with:	Package Description
Ion Exchange		
0045001	TOYOPEARL GigaCap S-650M	8 × 200 µL
0045002	TOYOPEARL GigaCap S-650M	8 × 600 µL
0045003	TOYOPEARL GigaCap Q-650M	8 × 200 µL
0045004	TOYOPEARL GigaCap Q-650M	8 × 600 µL
0045005	TOYOPEARL GigaCap CM-650M	8 × 200 µL
0045006	TOYOPEARL GigaCap CM-650M	8 × 600 µL
0045011	TOYOPEARL Q-600C AR	8 × 200 µL
0045012	TOYOPEARL Q-600C AR	8 × 600 µL
Hydrophobic Interaction		
0045031	TOYOPEARL Phenyl-600M	8 × 200 µL
0045032	TOYOPEARL Phenyl-600M	8 × 600 µL
0045033	TOYOPEARL Butyl-600M	8 × 200 µL
0045034	TOYOPEARL Butyl-600M	8 × 600 µL
0045035	TOYOPEARL PPG-600M	8 × 200 µL
0045036	TOYOPEARL PPG-600M	8 × 600 µL
0045037	TOYOPEARL Phenyl-650M	8 × 200 µL
0045038	TOYOPEARL Phenyl-650M	8 × 600 µL
Mixed-Mode		
0045051	TOYOPEARL MX-Trp-650M	8 × 200 µL
0045052	TOYOPEARL MX-Trp-650M	8 × 600 µL
Protein A		
0045061	TOYOPEARL AF-rProtein A-650F	8 × 200 µL
0045062	TOYOPEARL AF-rProtein A-650F	8 × 600 µL
Accessories		
0045099	Array plate	

About: TOYOPEARL and TSKgel LabPak media

LabPak products are multi-milliliter containers of TOYOPEARL and TSKgel bulk media products. Typically they contain 3 or 4 different ligand types offered for a particular chromatography mode.

They are useful for developmental engineers who wish to familiarize themselves with resin physical properties in different buffer systems:

- slurry and reslurry mechanics
- resin handling during column packing
- mechanical strength relative to agarose
- degree of compressibility
- flow adaptor regimen

The larger resin amounts in LabPak products allow the packing of wider ID and longer columns than available in the ToyoScreen products. This helps the developmental chemist or engineer to better measure under actual packing conditions the following properties:

- dynamic binding capacity
- selectivity
- column efficiency

Ordering Information - TOYOPEARL LabPak media

Part #	Description	Package Description
Size Exclusion		
0019820	SECPAK HP (HW-40, 50, 55, 65S), 30 µm	4 × 150 mL
0019821	SECPAK LMW (HW-40, 50, 55F), 45 µm	3 × 150 mL
0019819	SECPAK HMW (HW-55, 65, 75F), 45 µm	3 × 150 mL
Ion Exchange		
0019817	IEXPAK HP (DEAE-650S, SP-650S, CM-650S, SuperQ-650S), 35 µm	4 × 25 mL
0043210	AIEXPAK (GigaCap Q-650M, SuperQ-650M, Q-600C AR) , 65/75/100 µm	3 × 25 mL
0043220	CIEXPAK (GigaCap CM-650M, GigaCap S-650M, SP-550C), 75/100 µm	3 × 25 mL
Hydrophobic Interaction		
0043150	HICPAK HP (Ether, Phenyl, Butyl-650S), 35 µm	3 × 25 mL
0019806	HICPAK (Ether, Phenyl, Butyl-650M), 65 µm	3 × 25 mL
0043125	HICPAK-C (Phenyl, Butyl, Hexyl-650C), 100 µm	3 × 25 mL
Affinity		
0043400	AFFIPAK ACT (AF-Epoxy, Tresyl-650M), 65 µm	2 × 5 g*
0043410	AFFIPAK (AF-Amino, Carboxyl, Formyl-650 M), 65 µm	3 × 10 mL

*1 g is approximately 3.5 mL

Ordering Information - TSKgel LabPak media

Part #	Description	Package Description
Ion Exchange		
0043380	IEXPAK PW (DEAE-5PW, SP-5PW, SuperQ-5PW), 20 µm	3 × 25 mL
0043280	IEXPAK PW (DEAE-5PW, SP-5PW, SuperQ-5PW), 30 µm	3 × 25 mL
Hydrophobic Interaction		
0043278	HICPAK PW (Ether-5PW, Phenyl-5PW) , 20 µm	2 × 25 mL
0043175	HICPAK PW (Ether-5PW, Phenyl-5PW) , 30 µm	2 × 25 mL

About: Resin Seeker 96-well plate kits

Resin Seeker 96-well plates are disposable filter plates packed with TOYOPEARL and/or TOYOPEARL GigaCap® resins and are available in several configurations for ion exchange, HIC and mixed-mode chromatography. Resin Seeker 96-well plates can be used to screen multiple steps of the purification process including binding, wash and elution conditions in addition to resin selectivity, binding kinetics, purity and recovery of your target molecule. Resin Seeker plates can be operated manually using a multi-channel pipette or in an automated system designed for high throughput screening in a 96-well plate format.

These 96-well plate kits are manufactured by Orochem and sold by Tosoh Bioscience. All components necessary to run an experiment are included in each kit: a wash plate and collection plate are included in each 96-well plate package.

All TOYOPEARL and/or TOYOPEARL GigaCap resins used in the Resin Seeker 96-well plates are also available in ToyoScreen pre-packed columns and as bulk media. This allows seamless scale-up and process optimization once resin screening is complete.

Ordering Information - Resin Seeker 96-well plate kits

Part #	Description	Package Description
OC41MDAEX-96	AIEX 96-well plate kits	Mixed anion exchange plate (20 µL resin beds)
OC41MDCEX-96	CIEX 96-well plate kits	Mixed cation exchange plate (20 µL resin beds)
OC41MDHIC-96	HIC 96-well plate kits	Mixed hydrophobic interaction plate (20 µL resin beds)
OC41MDTRP-96	MMC 96-well plate kits	TOYOPEARL MX-Trp-650M plate (20 µL resin beds)

About: TOYOPEARL and TSKgel bulk media (<1 L)

TOYOPEARL HW and TSKgel PW size exclusion resins are the foundation for all ion exchange, hydrophobic interaction, mixed-mode and affinity process media products from Tosoh Bioscience. After an initial polymerization reaction to create the designated particle size and pore size, the resin surface is chemically modified to create the desired final product.

TOYOPEARL HW products are hydroxylated methacrylic polymer resins. Toyopearl HW beads are made commercially in many different pore sizes and particle diameters (30 µm and larger). TSKgel PW products are hydroxylated methacrylic polymer resins and are made commercially in many different pore sizes and particle diameters (30 µm and smaller).

Ordering Information - TOYOPEARL and TSKgel bulk media (< 1 L)

Part #	Description	Matrix	Container size
Size Exclusion			
0019809	TOYOPEARL HW-40S, 30 µm	polymer	150 mL
0007451	TOYOPEARL HW-40S, 30 µm		250 mL
0007447	TOYOPEARL HW-40S, 30 µm		500 mL
0019808	TOYOPEARL HW-40F, 45 µm		150 mL
0007448	TOYOPEARL HW-40F, 45 µm		500 mL
0019807	TOYOPEARL HW-40C, 75 µm		150 mL
0007449	TOYOPEARL HW-40C, 75 µm		500 mL
0007450	TOYOPEARL HW-40EC, 200 µm		500 mL
0019811	TOYOPEARL HW-50S, 30 µm		150 mL
0007455	TOYOPEARL HW-50S, 30 µm		250 mL
0007452	TOYOPEARL HW-50S, 30 µm		500 mL
0019810	TOYOPEARL HW-50F, 45 µm		150 mL
0007453	TOYOPEARL HW-50F, 45 µm		500 mL
0019813	TOYOPEARL HW-55S, 30 µm		150 mL
0007459	TOYOPEARL HW-55S, 30 µm		250 mL
0007456	TOYOPEARL HW-55S, 30 µm		500 mL
0019812	TOYOPEARL HW-55F, 45 µm		150 mL
0007457	TOYOPEARL HW-55F, 45 µm		500 mL
0019815	TOYOPEARL HW-65S, 30 µm		150 mL
0007467	TOYOPEARL HW-65S, 30 µm		250 mL
0007464	TOYOPEARL HW-65S, 30 µm		500 mL
0019814	TOYOPEARL HW-65F, 45 µm		150 mL
0007465	TOYOPEARL HW-65F, 45 µm		500 mL
0021481	TOYOPEARL HW-65C, 75 µm		150 mL
0007466	TOYOPEARL HW-65C, 75 µm		500 mL
0007471	TOYOPEARL HW-75S, 30 µm		250 mL
0007468	TOYOPEARL HW-75S, 30 µm		500 mL
0019816	TOYOPEARL HW-75F, 45 µm		150 mL
0007469	TOYOPEARL HW-75F, 45 µm		500 mL
Anion Exchange			
0043381	TSKgel DEAE-5PW, 20 µm	polymer	25 mL
0014710	TSKgel DEAE-5PW, 20 µm		250 mL
0043281	TSKgel DEAE-5PW, 30 µm		25 mL
0014712	TSKgel DEAE-5PW, 30 µm		250 mL
0043383	TSKgel SuperQ-5PW, 20 µm		25 mL

Part #	Description	Matrix	Container size
0018535	TSKgel SuperQ-5PW, 20 µm	polymer	250 mL
0043283	TSKgel SuperQ-5PW, 30 µm		25 mL
0018536	TSKgel SuperQ-5PW, 30 µm		250 mL
0019804	TOYOPEARL DEAE-650S, 35 µm		25 mL
0007472	TOYOPEARL DEAE-650S, 35 µm		250 mL
0043201	TOYOPEARL DEAE-650M, 65 µm		100 mL
0007473	TOYOPEARL DEAE-650M, 65 µm		250 mL
0022865	TOYOPEARL GigaCap DEAE-650M, 75 µm		100 mL
0022866	TOYOPEARL GigaCap DEAE-650M, 75 µm		250 mL
0007988	TOYOPEARL DEAE-650C, 100 µm		250 mL
0022881	TOYOPEARL GigaCap Q-650S, 35 µm		25 mL
0022882	TOYOPEARL GigaCap Q-650S, 35 µm		250 mL
0021854	TOYOPEARL GigaCap Q-650M, 75 µm		100 mL
0021855	TOYOPEARL GigaCap Q-650M, 75 µm		250 mL
0021985	TOYOPEARL Q-600C AR, 100 µm		100 mL
0021986	TOYOPEARL Q-600C AR, 100 µm		250 mL
0043271	TOYOPEARL QAE-550C, 100 µm		100 mL
0014026	TOYOPEARL QAE-550C, 100 µm		250 mL
0019823	TOYOPEARL SuperQ-650S, 35 µm		25 mL
0017223	TOYOPEARL SuperQ-650S, 35 µm		250 mL
0043205	TOYOPEARL SuperQ-650M, 65 µm		100 mL
0017227	TOYOPEARL SuperQ-650M, 65 µm		250 mL
0043275	TOYOPEARL SuperQ-650C, 100 µm		100 mL
0017231	TOYOPEARL SuperQ-650C, 100 µm		250 mL
Cation Exchange			
0021976	TSKgel SP-3PW, 30 µm	polymer	25 mL
0021977	TSKgel SP-3PW, 30 µm		250 mL
0043382	TSKgel SP-5PW, 20 µm		25 mL
0014714	TSKgel SP-5PW, 20 µm		250 mL
0043282	TSKgel SP-5PW, 30 µm		25 mL
0014716	TSKgel SP-5PW, 30 µm		250 mL
0019803	TOYOPEARL CM-650S, 35 µm		25 mL
0007474	TOYOPEARL CM-650S, 35 µm		250 mL
0043203	TOYOPEARL CM-650M, 65 µm		100 mL
0007475	TOYOPEARL CM-650M, 65 µm		250 mL
0007991	TOYOPEARL CM-650C, 100 µm		250 mL
0022875	TOYOPEARL GigaCap S-650S, 35 µm		25 mL
0022876	TOYOPEARL GigaCap S-650S, 35 µm		250 mL
0021833	TOYOPEARL GigaCap S-650M, 75 µm		100 mL
0021834	TOYOPEARL GigaCap S-650M, 75 µm		250 mL
0021946	TOYOPEARL GigaCap CM-650M, 75 µm		100 mL
0021947	TOYOPEARL GigaCap CM-650M, 75 µm		250 mL
0021804	TOYOPEARL MegaCap II SP-550EC, >100 µm		100 mL

Part #	Description	Matrix	Container size
0021805	TOYOPEARL MegaCap II SP-550EC, >100 µm	polymer	250 mL
0019822	TOYOPEARL SP-650S, 35 µm		25 mL
0008437	TOYOPEARL SP-650S, 35 µm		250 mL
0043202	TOYOPEARL SP-650M, 65 µm		100 mL
0007997	TOYOPEARL SP-650M, 65 µm		250 mL
0007994	TOYOPEARL SP-650C, 100 µm		250 mL
0043272	TOYOPEARL SP-550C, 100 µm		100 mL
0014028	TOYOPEARL SP-550C, 100 µm		250 mL
Hydrophobic Interaction			
0043276	TSKgel Ether-5PW, 20 µm	polymer	25 mL
0016052	TSKgel Ether-5PW, 20 µm		250 mL
0043176	TSKgel Ether-5PW, 30 µm		25 mL
0016050	TSKgel Ether-5PW, 30 µm		250 mL
0043277	TSKgel Phenyl-5PW, 20 µm		25 mL
0014718	TSKgel Phenyl-5PW, 20 µm		250 mL
0043177	TSKgel Phenyl-5PW, 30 µm		25 mL
0014720	TSKgel Phenyl-5PW, 30 µm		250 mL
0019955	TOYOPEARL SuperButyl-550C, 100 µm		25 mL
0019956	TOYOPEARL SuperButyl-550C, 100 µm		100 mL
0021448	TOYOPEARL Butyl-600M, 65 µm		25 mL
0021449	TOYOPEARL Butyl-600M, 65 µm		100 mL
0043153	TOYOPEARL Butyl-650S, 35 µm		25 mL
0007476	TOYOPEARL Butyl-650S, 35 µm		100 mL
0019802	TOYOPEARL Butyl-650M, 65 µm		25 mL
0007477	TOYOPEARL Butyl-650M, 65 µm		100 mL
0043127	TOYOPEARL Butyl-650C, 100 µm		25 mL
0007478	TOYOPEARL Butyl-650C, 100 µm		100 mL
0043151	TOYOPEARL Ether-650S, 35 µm		25 mL
0016172	TOYOPEARL Ether-650S, 35 µm		100 mL
0019805	TOYOPEARL Ether-650M , 65 µm		25 mL
0016173	TOYOPEARL Ether-650M , 65 µm		100 mL
0044465	TOYOPEARL Hexyl-650C, 100 µm		25 mL
0019026	TOYOPEARL Hexyl-650C, 100 µm		100 mL
0021887	TOYOPEARL Phenyl-600M, 65 µm		25 mL
0021888	TOYOPEARL Phenyl-600M, 65 µm		100 mL
0043152	TOYOPEARL Phenyl-650S, 35 µm		25 mL
0014477	TOYOPEARL Phenyl-650S, 35 µm		100 mL
0019818	TOYOPEARL Phenyl-650M, 65 µm		25 mL
0014478	TOYOPEARL Phenyl-650M, 65 µm		100 mL
0043126	TOYOPEARL Phenyl-650C, 100 µm		25 mL
0014479	TOYOPEARL Phenyl-650C, 100 µm		100 mL
0021301	TOYOPEARL PPG-600M, 65 µm		25 mL
0021302	TOYOPEARL PPG-600M, 65 µm		100 mL

Part #	Description	Matrix	Container size
Mixed-Mode			
0022817	TOYOPEARL MX-Trp-650M, 75 µm	polymer	25 mL
0022818	TOYOPEARL MX-Trp-650M, 75 µm		100 mL
Affinity			
0016208	TSKgel Tresyl-5PW, 10 µm	polymer	2 g*
0043411	TOYOPEARL AF-Amino-650M, 65 µm		10 mL
0008002	TOYOPEARL AF-Amino-650M, 65 µm		25 mL
0008039	TOYOPEARL AF-Amino-650M, 65 µm		100 mL
0019688	TOYOPEARL AF-Blue HC-650M, 65 µm		25 mL
0019689	TOYOPEARL AF-Blue HC-650M, 65 µm		100 mL
0043412	TOYOPEARL AF-Carboxy-650M, 65 µm		10 mL
0008006	TOYOPEARL AF-Carboxy-650M, 65 µm		25 mL
0008041	TOYOPEARL AF-Carboxy-650M, 65 µm		100 mL
0014475	TOYOPEARL AF-Chelate-650M, 65 µm		25 mL
0019800	TOYOPEARL AF-Chelate-650M, 65 µm		100 mL
0043402	TOYOPEARL AF-Epoxy-650M, 65 µm		5 g
0008000	TOYOPEARL AF-Epoxy-650M, 65 µm		10 g
0008038	TOYOPEARL AF-Epoxy-650M, 65 µm		100 g
0043413	TOYOPEARL AF-Formyl-650M, 65 µm		10 mL
0008004	TOYOPEARL AF-Formyl-650M, 65 µm		25 mL
0008040	TOYOPEARL AF-Formyl-650M, 65 µm		100 mL
0020030	TOYOPEARL AF-Heparin-HC650M, 65 µm		10 mL
0020031	TOYOPEARL AF-Heparin-HC650M, 65 µm		100 mL
0008651	TOYOPEARL AF-Red-650ML, 65 µm		25 mL
0019801	TOYOPEARL AF-Red-650ML, 65 µm		100 mL
0014471	TOYOPEARL AF-Tresyl-650M, 65 µm		5 g*
0014472	TOYOPEARL AF-Tresyl-650M, 65 µm		100 g*
0014905	TOYOPEARL AF-Tresyl-650M, 65 µm		200 g*
Protein A			
0022803	TOYOPEARL AF-rProtein A-650F, 45 µm	polymer	10 mL
0022804	TOYOPEARL AF-rProtein A-650F, 45 µm		25 mL
0022805	TOYOPEARL AF-rProtein A-650F, 45 µm		100 mL
0023425	TOYOPEARL AF-rProtein A HC-650F, 45 µm		10 mL
0023426	TOYOPEARL AF-rProtein A HC-650F, 45 µm		25 mL
0023427	TOYOPEARL AF-rProtein A HC-650F, 45 µm		100 mL

*1 g is approximately 3.5 mL

About: TOYOPEARL protein A ELISA kits

ELISA (enzyme-linked immunosorbent assay) kits are available for TOYOPEARL AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F resins. The TOYOPEARL ELISA kits are used for the quantitation of leached protein A ligand present in eluted product.

Please note that these kits are specifically prepared for TOYOPEARL AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F resins respectively. Test kits for other commercially available protein A products may not work properly for these TOYOPEARL protein A resins.

Ordering Information - TOYOPEARL protein A ELISA kits

Part #	Description
0022815	TOYOPEARL AF-rProtein A-650F ELISA
0023433	TOYOPEARL AF-rProtein A HC-650F ELISA


AF-rProtein A-650F
ToyoScreen

Part Number Index

Bulk Media

Part #	Description	Container size	Page
0007447	TOYOPEARL HW-40S, 30 µm	500 mL	4
0007448	TOYOPEARL HW-40F, 45 µm	500 mL	4
0007449	TOYOPEARL HW-40C, 75 µm	500 mL	4
0007450	TOYOPEARL HW-40EC, 200 µm	500 mL	4
0007451	TOYOPEARL HW-40S, 30 µm	250 mL	4
0007452	TOYOPEARL HW-50S, 30 µm	500 mL	4
0007453	TOYOPEARL HW-50F, 45 µm	500 mL	4
0007455	TOYOPEARL HW-50S, 30 µm	250 mL	4
0007456	TOYOPEARL HW-55S, 30 µm	500 mL	4
0007457	TOYOPEARL HW-55F, 45 µm	500 mL	4
0007459	TOYOPEARL HW-55S, 30 µm	250 mL	4
0007464	TOYOPEARL HW-65S, 30 µm	500 mL	4
0007465	TOYOPEARL HW-65F, 45 µm	500 mL	4
0007466	TOYOPEARL HW-65C, 75 µm	500 mL	4
0007467	TOYOPEARL HW-65S, 30 µm	250 mL	4
0007468	TOYOPEARL HW-75S, 30 µm	500 mL	4
0007469	TOYOPEARL HW-75F, 45 µm	500 mL	4
0007471	TOYOPEARL HW-75S, 30 µm	250 mL	4
0007472	TOYOPEARL DEAE-650S, 35 µm	250 mL	14
0007473	TOYOPEARL DEAE-650M, 65 µm	250 mL	14
0007474	TOYOPEARL CM-650S, 35 µm	250 mL	14
0007475	TOYOPEARL CM-650M, 65 µm	250 mL	14
0007476	TOYOPEARL Butyl-650S, 35 µm	100 mL	38
0007477	TOYOPEARL Butyl-650M, 65 µm	100 mL	38
0007478	TOYOPEARL Butyl-650C, 100 µm	100 mL	38
0007967	TOYOPEARL HW-40S, 30 µm	5 L	4
0007968	TOYOPEARL HW-40F, 45 µm	5 L	4
0007969	TOYOPEARL HW-40C, 75 µm	5 L	4
0007970	TOYOPEARL HW-40EC, 200µm	5 L	4
0007971	TOYOPEARL CM-650S, 35 µm	5 L	14
0007972	TOYOPEARL CM-650M, 65 µm	5 L	14
0007973	TOYOPEARL DEAE-650S, 35 µm	5 L	14
0007974	TOYOPEARL DEAE-650M, 65 µm	5 L	14
0007975	TOYOPEARL Butyl-650S, 35 µm	5 L	38
0007976	TOYOPEARL Butyl-650M, 65 µm	5 L	38
0007977	TOYOPEARL Butyl-650C, 100 µm	5 L	38
0007988	TOYOPEARL DEAE-650C, 100 µm	250 mL	14
0007989	TOYOPEARL DEAE-650C, 100 µm	5 L	14
0007991	TOYOPEARL CM-650C, 100 µm	250 mL	14
0007992	TOYOPEARL CM-650C, 100 µm	5 L	14
0007994	TOYOPEARL SP-650C, 100 µm	250 mL	14
0007995	TOYOPEARL SP-650C, 100 µm	5 L	14
0007997	TOYOPEARL SP-650M, 65 µm	250 mL	14
0007998	TOYOPEARL SP-650M, 65 µm	5 L	14
0008000	TOYOPEARL AF-Epoxy-650M, 65 µm	10 g	62
0008002	TOYOPEARL AF-Amino-650M, 65 µm	25 mL	62
0008004	TOYOPEARL AF-Formyl-650M, 65 µm	25 mL	62
0008006	TOYOPEARL AF-Carboxy-650M, 65 µm	25 mL	62
0008038	TOYOPEARL AF-Epoxy-650M, 65 µm	100 g	62
0008039	TOYOPEARL AF-Amino-650M, 65 µm	100 mL	62
0008040	TOYOPEARL AF-Formyl-650M, 65 µm	100 mL	62
0008041	TOYOPEARL AF-Carboxy-650M, 65 µm	100 mL	62
0008059	TOYOPEARL HW-50S, 30 µm	5 L	4
0008060	TOYOPEARL HW-50F, 45 µm	5 L	4
0008062	TOYOPEARL HW-55S, 30 µm	5 L	4
0008063	TOYOPEARL HW-55F, 45 µm	5 L	4
0008068	TOYOPEARL HW-65S, 30 µm	5 L	4
0008069	TOYOPEARL HW-65F, 45 µm	5 L	4
0008070	TOYOPEARL HW-65C, 75 µm	5 L	4
0008071	TOYOPEARL HW-75S, 30 µm	5 L	4
0008072	TOYOPEARL HW-75F, 45 µm	5 L	4
0008437	TOYOPEARL SP-650S, 35 µm	250 mL	14
0008438	TOYOPEARL SP-650S, 35 µm	5 L	14
0008651	TOYOPEARL AF-Red-650ML, 65 µm	25 mL	62
0014026	TOYOPEARL QAE-550C, 100 µm	250 mL	14
0014027	TOYOPEARL QAE-550C, 100 µm	5 L	14
0014028	TOYOPEARL SP-550C, 100 µm	250 mL	14
0014029	TOYOPEARL SP-550C, 100 µm	5 L	14
0014471	TOYOPEARL AF-Tresyl-650M, 65 µm	5 g	62
0014472	TOYOPEARL AF-Tresyl-650M, 65 µm	100 g	62
0014475	TOYOPEARL AF-Chelate-650M, 65 µm	25 mL	62
0014477	TOYOPEARL Phenyl-650S, 35 µm	100 mL	38
0014478	TOYOPEARL Phenyl-650M, 65 µm	100 mL	38
0014479	TOYOPEARL Phenyl-650C, 100 µm	100 mL	38
0014681	TOYOPEARL HW-40S, 30 µm	1 L	4
0014682	TOYOPEARL HW-40F, 45 µm	1 L	4
0014683	TOYOPEARL HW-40C, 75 µm	1 L	4
0014684	TOYOPEARL HW-50S, 30 µm	1 L	4
0014685	TOYOPEARL HW-50F, 45 µm	1 L	4
0014686	TOYOPEARL HW-55S, 30 µm	1 L	4
0014687	TOYOPEARL HW-55F, 45 µm	1 L	4
0014688	TOYOPEARL HW-65S, 30 µm	1 L	4
0014689	TOYOPEARL HW-65F, 45 µm	1 L	4
0014690	TOYOPEARL HW-65C, 75 µm	1 L	4
0014691	TOYOPEARL HW-75F, 45 µm	1 L	4
0014692	TOYOPEARL DEAE-650S, 35 µm	1 L	14
0014693	TOYOPEARL DEAE-650M, 65 µm	1 L	14
0014694	TOYOPEARL DEAE-650C, 100 µm	1 L	14
0014695	TOYOPEARL CM-650S, 35 µm	1 L	14
0014696	TOYOPEARL CM-650M, 65 µm	1 L	14
0014697	TOYOPEARL CM-650C, 100 µm	1 L	14
0014698	TOYOPEARL SP-650S, 35 µm	1 L	14
0014699	TOYOPEARL SP-650M, 65 µm	1 L	14
0014700	TOYOPEARL SP-650C, 100 µm	1 L	14
0014701	TOYOPEARL Butyl-650S, 35 µm	1 L	38
0014702	TOYOPEARL Butyl-650M, 65 µm	1 L	38
0014703	TOYOPEARL Butyl-650C, 100 µm	1 L	38
0014704	TOYOPEARL QAE-550C, 100 µm	1 L	14
0014705	TOYOPEARL SP-550C, 100 µm	1 L	14
0014710	TSKgel DEAE-5PW, 20 µm	250 mL	14
0014711	TSKgel DEAE-5PW, 20 µm	1 L	14
0014712	TSKgel DEAE-5PW, 30 µm	250 mL	14
0014713	TSKgel DEAE-5PW, 30 µm	1 L	14
0014714	TSKgel SP-5PW, 20 µm	250 mL	14
0014715	TSKgel SP-5PW, 20 µm	1 L	14
0014716	TSKgel SP-5PW, 30 µm	250 mL	14
0014717	TSKgel SP-5PW, 30 µm	1 L	14
0014718	TSKgel Phenyl-5PW, 20 µm	250 mL	38
0014719	TSKgel Phenyl-5PW, 20 µm	1 L	38
0014720	TSKgel Phenyl-5PW, 30 µm	250 mL	38
0014721	TSKgel Phenyl-5PW, 30 µm	1 L	38
0014783	TOYOPEARL Phenyl-650M, 65 µm	1 L	38
0014784	TOYOPEARL Phenyl-650S, 35 µm	1 L	38
0014785	TOYOPEARL Phenyl-650C, 100 µm	1 L	38
0014905	TOYOPEARL AF-Tresyl-650M, 65 µm	200 g	62
0014906	TOYOPEARL AF-Tresyl-650M, 65 µm	1 L	62
0014907	TOYOPEARL AF-Chelate-650M, 65 µm	1 L	62
0014908	TOYOPEARL AF-Chelate-650M, 65 µm	5 L	62
0014935	TOYOPEARL Phenyl-650S, 35 µm	5 L	38
0014943	TOYOPEARL Phenyl-650M, 65 µm	5 L	38
0014944	TOYOPEARL Phenyl-650C, 100 µm	5 L	38
0016050	TSKgel Ether-5PW, 30 µm	250 mL	38
0016051	TSKgel Ether-5PW, 30 µm	1 L	38
0016052	TSKgel Ether-5PW, 20 µm	250 mL	38
0016053	TSKgel Ether-5PW, 20 µm	1 L	38
0016172	TOYOPEARL Ether-650S, 35 µm	100 mL	38
0016173	TOYOPEARL Ether-650M , 65 µm	100 mL	38
0016174	TOYOPEARL Ether-650S, 35 µm	1 L	38
0016175	TOYOPEARL Ether-650M , 65 µm	1 L	38
0016176	TOYOPEARL Ether-650S, 35 µm	5 L	38
0016177	TOYOPEARL Ether-650M , 65 µm	5 L	38
0017210	TSKgel Phenyl-5PW, 30 µm	5 L	38
0017223	TOYOPEARL SuperQ-650S, 35 µm	250 mL	14
0017224	TOYOPEARL SuperQ-650S, 35 µm	1 L	14
0017225	TOYOPEARL SuperQ-650S, 35 µm	5 L	14
0017227	TOYOPEARL SuperQ-650M, 65 µm	250 mL	14
0017228	TOYOPEARL SuperQ-650M, 65 µm	1 L	14
0017229	TOYOPEARL SuperQ-650M, 65 µm	5 L	14
0017231	TOYOPEARL SuperQ-650C, 100 µm	250 mL	14
0017232	TOYOPEARL SuperQ-650C, 100 µm	1 L	14
0017233	TOYOPEARL SuperQ-650C, 100 µm	5 L	14
0017396	TOYOPEARL AF-Formyl-650M, 65 µm	1 L	62
0017397	TOYOPEARL AF-Formyl-650M, 65 µm	5 L	62
0018074	TOYOPEARL AF-Amino-650M, 65 µm	1 L	62
0018315	TOYOPEARL AF-Epoxy-650M, 65 µm	1 L	62
0018316	TOYOPEARL AF-Amino-650M, 65 µm	5 L	62
0018355	TOYOPEARL Butyl-650M, 65 µm	50 L	38
0018364	TOYOPEARL Phenyl-650M, 65 µm	50 L	38
0018365	TOYOPEARL QAE-550C, 100 µm	50 L	14
0018366	TOYOPEARL SP-550C, 100 µm	50 L	14
0018367	TOYOPEARL DEAE-650M, 65 µm	50 L	14
0018368	TOYOPEARL HW-50F, 45 µm	50 L	4
0018369	TOYOPEARL SP-650M, 65 µm	50 L	14
0018370	TSKgel DEAE-5PW, 30 µm	5 L	14
0018371	TOYOPEARL AF-Tresyl-650M, 65 µm	5 L	62
0018384	TSKgel SP-5PW, 30 µm	5 L	14
0018435	TSKgel SP-5PW, 20 µm	5 L	14
0018436	TSKgel DEAE-5PW, 20 µm	5 L	14

Part #	Description	Container size	Page
0018437	TSKgel Ether-5PW, 20 µm	5 L	38
0018438	TSKgel Phenyl-5PW, 20 µm	5 L	38
0018439	TSKgel Ether-5PW, 30 µm	5 L	38
0018535	TSKgel SuperQ-5PW, 20 µm	250 mL	14
0018536	TSKgel SuperQ-5PW, 30 µm	250 mL	14
0018546	TSKgel SuperQ-5PW, 20 µm	1 L	14
0018547	TSKgel SuperQ-5PW, 20 µm	5 L	14
0018548	TSKgel SuperQ-5PW, 30 µm	1 L	14
0018549	TSKgel SuperQ-5PW, 30 µm	5 L	14
0018826	TOYOPEARL Butyl-650S, 35 µm	50 L	38
0018827	TOYOPEARL AF-Carboxy-650M, 65 µm	1 L	62
0018828	TOYOPEARL AF-Carboxy-650M, 65 µm	5 L	62
0019026	TOYOPEARL Hexyl-650C, 100 µm	100 mL	38
0019027	TOYOPEARL Hexyl-650C, 100 µm	1 L	38
0019028	TOYOPEARL Hexyl-650C, 100 µm	5 L	38
0019329	TOYOPEARL CM-650C, 100 µm	50 L	14
0019679	TOYOPEARL SuperQ-650S, 35 µm	50 L	14
0019688	TOYOPEARL AF-Blue HC-650M, 65 µm	25 mL	62
0019689	TOYOPEARL AF-Blue HC-650M, 65 µm	100 mL	62
0019690	TOYOPEARL AF-Blue HC-650M, 65 µm	1 L	62
0019691	TOYOPEARL AF-Blue HC-650M, 65 µm	5 L	62
0019800	TOYOPEARL AF-Chelate-650M, 65 µm	100 mL	62
0019801	TOYOPEARL AF-Red-650ML, 65 µm	100 mL	62
0019802	TOYOPEARL Butyl-650M, 65 µm	25 mL	38
0019803	TOYOPEARL CM-650S, 35 µm	25 mL	14
0019804	TOYOPEARL DEAE-650S, 35 µm	25 mL	14
0019805	TOYOPEARL Ether-650M , 65 µm	25 mL	38
0019807	TOYOPEARL HW-40C, 75 µm	150 mL	4
0019808	TOYOPEARL HW-40F, 45 µm	150 mL	4
0019809	TOYOPEARL HW-40S, 30 µm	150 mL	4
0019810	TOYOPEARL HW-50F, 45 µm	150 mL	4
0019811	TOYOPEARL HW-50S, 30 µm	150 mL	4
0019812	TOYOPEARL HW-55F, 45 µm	150 mL	4
0019813	TOYOPEARL HW-55S, 30 µm	150 mL	4
0019814	TOYOPEARL HW-65F, 45 µm	150 mL	4
0019815	TOYOPEARL HW-65S, 30 µm	150 mL	4
0019816	TOYOPEARL HW-75F, 45 µm	150 mL	4
0019818	TOYOPEARL Phenyl-650M, 65 µm	25 mL	38
0019822	TOYOPEARL SP-650S, 35 µm	25 mL	14
0019823	TOYOPEARL SuperQ-650S, 35 µm	25 mL	14
0019839	TOYOPEARL CM-650M, 65 µm	50 L	14
0019955	TOYOPEARL SuperButyl-550C, 100 µm	25 mL	38
0019956	TOYOPEARL SuperButyl-550C, 100 µm	100mL	38
0019957	TOYOPEARL SuperButyl-550C, 100 µm	1 L	38
0019958	TOYOPEARL SuperButyl-550C, 100 µm	5 L	38
0019959	TOYOPEARL SuperButyl-550C, 100 µm	50 L	38
0020030	TOYOPEARL AF-Heparin-HC650M, 65 µm	10 mL	62
0020031	TOYOPEARL AF-Heparin-HC650M, 65 µm	100 mL	62
0020032	TOYOPEARL AF-Heparin-HC650M, 65 µm	1 L	62
0020033	TOYOPEARL AF-Heparin-HC650M, 65 µm	5 L	62
0020891	TOYOPEARL Phenyl-600M, 65 µm	50 L	38
0021301	TOYOPEARL PPG-600M, 65 µm	25 mL	38
0021302	TOYOPEARL PPG-600M, 65 µm	100 mL	38
0021303	TOYOPEARL PPG-600M, 65 µm	1 L	38
0021304	TOYOPEARL PPG-600M, 65 µm	5 L	38
0021305	TOYOPEARL PPG-600M, 65 µm	50 L	38
0021311	TOYOPEARL SuperQ-650M, 65 µm	50 L	14
0021448	TOYOPEARL Butyl-600M, 65 µm	25 mL	38
0021449	TOYOPEARL Butyl-600M, 65 µm	100 mL	38
0021450	TOYOPEARL Butyl-600M, 65 µm	1 L	38
0021451	TOYOPEARL Butyl-600M, 65 µm	5 L	38
0021452	TOYOPEARL Butyl-600M, 65 µm	50 L	38
0021477	TOYOPEARL SP-650S, 35 µm	50 L	14
0021481	TOYOPEARL HW-65C, 75 µm	150 mL	4
0021482	TOYOPEARL HW-65C, 75 µm	50 L	4
0021483	TOYOPEARL DEAE-650S, 35 µm	50 L	14
0021484	TOYOPEARL HW-40C, 75 µm	50 L	4
0021804	TOYOPEARL MegaCap II SP-550EC, >100 µm	100 mL	14
0021805	TOYOPEARL MegaCap II SP-550EC, >100 µm	250 mL	14
0021806	TOYOPEARL MegaCap II SP-550EC, >100 µm	1 L	14
0021807	TOYOPEARL MegaCap II SP-550EC, >100 µm	5 L	14
0021807	TSKgel SP-5PW, 30 µm	50 L	14
0021808	TOYOPEARL MegaCap II SP-550EC, >100 µm	50 L	14
0021833	TOYOPEARL GigaCap S-650M, 75 µm	100 mL	14
0021834	TOYOPEARL GigaCap S-650M, 75 µm	250 mL	14
0021835	TOYOPEARL GigaCap S-650M, 75 µm	1 L	14
0021836	TOYOPEARL GigaCap S-650M, 75 µm	5 L	14
0021837	TOYOPEARL GigaCap S-650M, 75 µm	50 L	14
0021852	TOYOPEARL HW-65F, 45 µm	50 L	4
0021854	TOYOPEARL GigaCap Q-650M, 75 µm	100 mL	14
0021855	TOYOPEARL GigaCap Q-650M, 75 µm	250 mL	14
0021856	TOYOPEARL GigaCap Q-650M, 75 µm	1 L	14
0021857	TOYOPEARL GigaCap Q-650M, 75 µm	5 L	14
0021858	TOYOPEARL GigaCap Q-650M, 75 µm	50 L	14
0021887	TOYOPEARL Phenyl-600M, 65 µm	25 mL	38
0021888	TOYOPEARL Phenyl-600M, 65 µm	100 mL	38
0021889	TOYOPEARL Phenyl-600M, 65 µm	1 L	38
0021890	TOYOPEARL Phenyl-600M, 65 µm	5 L	38
0021918	TOYOPEARL HW-55F, 45 µm	50 L	4
0021919	TSKgel SuperQ-5PW, 20 µm	25 L	14
0021920	TSKgel SuperQ-5PW, 20 µm	50 L	14
0021946	TOYOPEARL GigaCap CM-650M, 75 µm	100 mL	14
0021947	TOYOPEARL GigaCap CM-650M, 75 µm	250 mL	14
0021948	TOYOPEARL GigaCap CM-650M, 75 µm	1 L	14
0021949	TOYOPEARL GigaCap CM-650M, 75 µm	5 L	14
0021950	TOYOPEARL GigaCap CM-650M, 75 µm	50 L	14
0021973	TOYOPEARL Hexyl-650C, 100 µm	50 L	38
0021976	TSKgel SP-3PW, 30 µm	25 mL	14
0021977	TSKgel SP-3PW, 30 µm	250 mL	14
0021978	TSKgel SP-3PW, 30 µm	1 L	14
0021979	TSKgel SP-3PW, 30 µm	5 L	14
0021980	TSKgel SP-3PW, 30 µm	50 L	14
0021985	TOYOPEARL Q-600C AR, 100 µm	100 mL	14
0021986	TOYOPEARL Q-600C AR, 100 µm	250 mL	14
0021987	TOYOPEARL Q-600C AR, 100 µm	1 L	14
0021988	TOYOPEARL Q-600C AR, 100 µm	5 L	14
0021989	TOYOPEARL Q-600C AR, 100 µm	50 L	14
0022803	TOYOPEARL AF-rProtein A-650F, 45 µm	10 mL	54
0022803	TOYOPEARL AF-rProtein A-650F, 45 µm	10 mL	54
0022804	TOYOPEARL AF-rProtein A-650F, 45 µm	25 mL	54
0022804	TOYOPEARL AF-rProtein A-650F, 45 µm	25 mL	54
0022805	TOYOPEARL AF-rProtein A-650F, 45 µm	100 mL	54
0022805	TOYOPEARL AF-rProtein A-650F, 45 µm	100 mL	54
0022806	TOYOPEARL AF-rProtein A-650F, 45 µm	1 L	54
0022807	TOYOPEARL AF-rProtein A-650F, 45 µm	5 L	54
0022808	TOYOPEARL AF-rProtein A-650F, 45 µm	50 L	54
0022817	TOYOPEARL MX-Trp-650M, 75 µm	25 mL	72
0022818	TOYOPEARL MX-Trp-650M, 75 µm	100 mL	72
0022819	TOYOPEARL MX-Trp-650M, 75 µm	1 L	72
0022820	TOYOPEARL MX-Trp-650M, 75 µm	5 L	72
0022826	TOYOPEARL Butyl-650C, 100 µm	50 L	38
0022853	TOYOPEARL DEAE-650C, 100 µm	50 L	14
0022865	TOYOPEARL GigaCap DEAE-650M, 75 µm	100 mL	14
0022866	TOYOPEARL GigaCap DEAE-650M, 75 µm	250 mL	14
0022867	TOYOPEARL GigaCap DEAE-650M, 75 µm	1 L	14
0022868	TOYOPEARL GigaCap DEAE-650M, 75 µm	5 L	14
0022869	TOYOPEARL GigaCap DEAE-650M, 75 µm	50 L	14
0022875	TOYOPEARL GigaCap S-650S, 35 µm	25 mL	14
0022876	TOYOPEARL GigaCap S-650S, 35 µm	250 mL	14
0022877	TOYOPEARL GigaCap S-650S, 35 µm	1 L	14
0022878	TOYOPEARL GigaCap S-650S, 35 µm	5 L	14
0022879	TOYOPEARL GigaCap S-650S, 35 µm	50 L	14
0022881	TOYOPEARL GigaCap Q-650S, 35 µm	25 mL	14
0022882	TOYOPEARL GigaCap Q-650S, 35 µm	250 mL	14
0022883	TOYOPEARL GigaCap Q-650S, 35 µm	1 L	14
0022884	TOYOPEARL GigaCap Q-650S, 35 µm	5 L	14
0022885	TOYOPEARL GigaCap Q-650S, 35 µm	50 L	14
0023425	TOYOPEARL AF-rProtein A HC-650F, 45 µm	10 mL	54
0023426	TOYOPEARL AF-rProtein A HC-650F, 45 µm	25 mL	54
0023427	TOYOPEARL AF-rProtein A HC-650F, 45 µm	100 mL	54
0023428	TOYOPEARL AF-rProtein A HC-650F, 45 µm	1 L	54
0023429	TOYOPEARL AF-rProtein A HC-650F, 45 µm	5 L	54
0023434	TOYOPEARL AF-rProtein A HC-650F, 45 µm	50 L	54
0042102	TOYOPEARL AF-Red-650ML, 65 µm	1 L	62
0043126	TOYOPEARL Phenyl-650C, 100 µm	25 mL	38
0043127	TOYOPEARL Butyl-650C, 100 µm	25 mL	38
0043151	TOYOPEARL Ether-650S, 35 µm	25 mL	38
0043152	TOYOPEARL Phenyl-650S, 35 µm	25 mL	38
0043153	TOYOPEARL Butyl-650S, 35 µm	25 mL	38
0043176	TSKgel Ether-5PW, 30 µm	25 mL	38
0043177	TSKgel Phenyl-5PW, 30 µm	25 mL	38
0043201	TOYOPEARL DEAE-650M, 65 µm	100 mL	14
0043202	TOYOPEARL SP-650M, 65 µm	100 mL	14
0043203	TOYOPEARL CM-650M, 65 µm	100 mL	14
0043205	TOYOPEARL SuperQ-650M, 65 µm	100 mL	14
0043271	TOYOPEARL QAE-550C, 100 µm	100 mL	14
0043272	TOYOPEARL SP-550C, 100 µm	100 mL	14
0043275	TOYOPEARL SuperQ-650C, 100 µm	100 mL	14
0043276	TSKgel Ether-5PW, 20 µm	25 mL	38
0043277	TSKgel Phenyl-5PW, 20 µm	25 mL	38
0043281	TSKgel DEAE-5PW, 30 µm	25 mL	14
0043282	TSKgel SP-5PW, 30 µm	25 mL	14

Process Development Columns

Process Development Accessories

ToyoScreen RoboColumns

TOYOPEARL LabPak Media

TSKgel LabPak Media

Resin Seeker 96-well plate kits

TOYOPEARL protein A ELISA kits

Description Index

Bulk Media

Description	Container Size	Part #	Page
TOYOPEARL HW-40S, 30 µm	1 L	0014681	4
TOYOPEARL HW-40S, 30 µm	5 L	0007967	4
TOYOPEARL HW-50F, 45 µm	150 mL	0019810	4
TOYOPEARL HW-50F, 45 µm	500 mL	0007453	4
TOYOPEARL HW-50F, 45 µm	1 L	0014685	4
TOYOPEARL HW-50F, 45 µm	5 L	0008060	4
TOYOPEARL HW-50F, 45 µm	50 L	0018368	4
TOYOPEARL HW-50S, 30 µm	150 mL	0019811	4
TOYOPEARL HW-50S, 30 µm	250 mL	0007455	4
TOYOPEARL HW-50S, 30 µm	500 mL	0007452	4
TOYOPEARL HW-50S, 30 µm	1 L	0014684	4
TOYOPEARL HW-50S, 30 µm	5 L	0008059	4
TOYOPEARL HW-55F, 45 µm	150 mL	0019812	4
TOYOPEARL HW-55F, 45 µm	500 mL	0007457	4
TOYOPEARL HW-55F, 45 µm	1 L	0014687	4
TOYOPEARL HW-55F, 45 µm	5 L	0008063	4
TOYOPEARL HW-55F, 45 µm	50 L	0021918	4
TOYOPEARL HW-55S, 30 µm	150 mL	0019813	4
TOYOPEARL HW-55S, 30 µm	250 mL	0007459	4
TOYOPEARL HW-55S, 30 µm	500 mL	0007456	4
TOYOPEARL HW-55S, 30 µm	1 L	0014686	4
TOYOPEARL HW-55S, 30 µm	5 L	0008062	4
TOYOPEARL HW-65C, 75 µm	150 mL	0021481	4
TOYOPEARL HW-65C, 75 µm	500 mL	0007466	4
TOYOPEARL HW-65C, 75 µm	1 L	0014690	4
TOYOPEARL HW-65C, 75 µm	5 L	0008070	4
TOYOPEARL HW-65C, 75 µm	50 L	0021482	4
TOYOPEARL HW-65F, 45 µm	150 mL	0019814	4
TOYOPEARL HW-65F, 45 µm	500 mL	0007465	4
TOYOPEARL HW-65F, 45 µm	1 L	0014689	4
TOYOPEARL HW-65F, 45 µm	5 L	0008069	4
TOYOPEARL HW-65F, 45 µm	50 L	0021852	4
TOYOPEARL HW-65S, 30 µm	150 mL	0019815	4
TOYOPEARL HW-65S, 30 µm	250 mL	0007467	4
TOYOPEARL HW-65S, 30 µm	500 mL	0007464	4
TOYOPEARL HW-65S, 30 µm	1 L	0014688	4
TOYOPEARL HW-65S, 30 µm	5 L	0008068	4
TOYOPEARL HW-75F, 45 µm	150 mL	0019816	4
TOYOPEARL HW-75F, 45 µm	500 mL	0007469	4
TOYOPEARL HW-75F, 45 µm	1 L	0014691	4
TOYOPEARL HW-75F, 45 µm	5 L	0008072	4
TOYOPEARL HW-75S, 30 µm	250 mL	0007471	4
TOYOPEARL HW-75S, 30 µm	500 mL	0007468	4
TOYOPEARL HW-75S, 30 µm	5 L	0008071	4
TOYOPEARL MegaCap II SP-550EC, >100 µm	100 mL	0021804	14
TOYOPEARL MegaCap II SP-550EC, >100 µm	250 mL	0021805	14
TOYOPEARL MegaCap II SP-550EC, >100 µm	1 L	0021806	14
TOYOPEARL MegaCap II SP-550EC, >100 µm	5 L	0021807	14
TOYOPEARL MegaCap II SP-550EC, >100 µm	50 L	0021808	14
TOYOPEARL MX-Trp-650M, 75 µm	25 mL	0022817	72
TOYOPEARL MX-Trp-650M, 75 µm	100 mL	0022818	72
TOYOPEARL MX-Trp-650M, 75 µm	1 L	0022819	72
TOYOPEARL MX-Trp-650M, 75 µm	5 L	0022820	72
TOYOPEARL Phenyl-600M, 65 µm	25 mL	0021887	38
TOYOPEARL Phenyl-600M, 65 µm	100 mL	0021888	38
TOYOPEARL Phenyl-600M, 65 µm	1 L	0021889	38
TOYOPEARL Phenyl-600M, 65 µm	5 L	0021890	38
TOYOPEARL Phenyl-600M, 65 µm	50 L	0020891	38
TOYOPEARL Phenyl-650C, 100 µm	25 mL	0043126	38
TOYOPEARL Phenyl-650C, 100 µm	100 mL	0014479	38
TOYOPEARL Phenyl-650C, 100 µm	1 L	0014785	38
TOYOPEARL Phenyl-650C, 100 µm	5 L	0014944	38
TOYOPEARL Phenyl-650M, 65 µm	25 mL	0019818	38
TOYOPEARL Phenyl-650M, 65 µm	100 mL	0014478	38
TOYOPEARL Phenyl-650M, 65 µm	1 L	0014783	38
TOYOPEARL Phenyl-650M, 65 µm	5 L	0014943	38
TOYOPEARL Phenyl-650M, 65 µm	50 L	0018364	38
TOYOPEARL Phenyl-650S, 35 µm	25 mL	0043152	38
TOYOPEARL Phenyl-650S, 35 µm	100 mL	0014477	38
TOYOPEARL Phenyl-650S, 35 µm	1 L	0014784	38
TOYOPEARL Phenyl-650S, 35 µm	5 L	0014935	38
TOYOPEARL PPG-600M, 65 µm	25 mL	0021301	38
TOYOPEARL PPG-600M, 65 µm	100 mL	0021302	38
TOYOPEARL PPG-600M, 65 µm	1 L	0021303	38
TOYOPEARL PPG-600M, 65 µm	5 L	0021304	38
TOYOPEARL PPG-600M, 65 µm	50 L	0021305	38
TOYOPEARL Q-600C AR, 100 µm	100 mL	0021985	14
TOYOPEARL Q-600C AR, 100 µm	250 mL	0021986	14
TOYOPEARL Q-600C AR, 100 µm	1 L	0021987	14
TOYOPEARL Q-600C AR, 100 µm	5 L	0021988	14
TOYOPEARL Q-600C AR, 100 µm	50 L	0021989	14

Description	Container Size	Part #	Page
TOYOPEARL QAE-550C, 100 µm	100 mL	0043271	14
TOYOPEARL QAE-550C, 100 µm	250 mL	0014026	14
TOYOPEARL QAE-550C, 100 µm	1 L	0014704	14
TOYOPEARL QAE-550C, 100 µm	5 L	0014027	14
TOYOPEARL QAE-550C, 100 µm	50 L	0018365	14
TOYOPEARL SP-550C, 100 µm	100 mL	0043272	14
TOYOPEARL SP-550C, 100 µm	250 mL	0014028	14
TOYOPEARL SP-550C, 100 µm	1 L	0014705	14
TOYOPEARL SP-550C, 100 µm	5 L	0014029	14
TOYOPEARL SP-550C, 100 µm	50 L	0018366	14
TOYOPEARL SP-650C, 100 µm	250 mL	0007994	14
TOYOPEARL SP-650C, 100 µm	1 L	0014700	14
TOYOPEARL SP-650C, 100 µm	5 L	0007995	14
TOYOPEARL SP-650M, 65 µm	100 mL	0043202	14
TOYOPEARL SP-650M, 65 µm	250 mL	0007997	14
TOYOPEARL SP-650M, 65 µm	1 L	0014699	14
TOYOPEARL SP-650M, 65 µm	5 L	0007998	14
TOYOPEARL SP-650M, 65 µm	50 L	0018369	14
TOYOPEARL SP-650S, 35 µm	25 mL	0019822	14
TOYOPEARL SP-650S, 35 µm	250 mL	0008437	14
TOYOPEARL SP-650S, 35 µm	1 L	0014698	14
TOYOPEARL SP-650S, 35 µm	5 L	0008438	14
TOYOPEARL SP-650S, 35 µm	50 L	0021477	14
TOYOPEARL SuperButyl-550C, 100 µm	25 mL	0019955	38
TOYOPEARL SuperButyl-550C, 100 µm	100 mL	0019956	38
TOYOPEARL SuperButyl-550C, 100 µm	1 L	0019957	38
TOYOPEARL SuperButyl-550C, 100 µm	5 L	0019958	38
TOYOPEARL SuperButyl-550C, 100 µm	50 L	0019959	38
TOYOPEARL SuperQ-650C, 100 µm	100 mL	0043275	14
TOYOPEARL SuperQ-650C, 100 µm	250 mL	0017231	14
TOYOPEARL SuperQ-650C, 100 µm	1 L	0017232	14
TOYOPEARL SuperQ-650C, 100 µm	5 L	0017233	14
TOYOPEARL SuperQ-650M, 65 µm	100 mL	0043205	14
TOYOPEARL SuperQ-650M, 65 µm	250 mL	0017227	14
TOYOPEARL SuperQ-650M, 65 µm	1 L	0017228	14
TOYOPEARL SuperQ-650M, 65 µm	5 L	0017229	14
TOYOPEARL SuperQ-650M, 65 µm	50 L	0021311	14
TOYOPEARL SuperQ-650S, 35 µm	25 mL	0019823	14
TOYOPEARL SuperQ-650S, 35 µm	250 mL	0017223	14
TOYOPEARL SuperQ-650S, 35 µm	1 L	0017224	14
TOYOPEARL SuperQ-650S, 35 µm	5 L	0017225	14
TOYOPEARL SuperQ-650S, 35 µm	50 L	0019679	14
TSKgel DEAE-5PW, 20 µm	25 mL	0043381	14
TSKgel DEAE-5PW, 20 µm	250 mL	0014710	14
TSKgel DEAE-5PW, 20 µm	1 L	0014711	14
TSKgel DEAE-5PW, 20 µm	5 L	0018436	14
TSKgel DEAE-5PW, 30 µm	25 mL	0043281	14
TSKgel DEAE-5PW, 30 µm	250 mL	0014712	14
TSKgel DEAE-5PW, 30 µm	1 L	0014713	14
TSKgel DEAE-5PW, 30 µm	5 L	0018370	14
TSKgel Ether-5PW, 20 µm	25 mL	0043276	38
TSKgel Ether-5PW, 20 µm	250 mL	0016052	38
TSKgel Ether-5PW, 20 µm	1 L	0016053	38
TSKgel Ether-5PW, 20 µm	5 L	0018437	38
TSKgel Ether-5PW, 30 µm	25 mL	0043176	38
TSKgel Ether-5PW, 30 µm	250 mL	0016050	38
TSKgel Ether-5PW, 30 µm	1 L	0016051	38
TSKgel Ether-5PW, 30 µm	5 L	0018439	38
TSKgel Phenyl-5PW, 20 µm	25 mL	0043277	38
TSKgel Phenyl-5PW, 20 µm	250 mL	0014718	38
TSKgel Phenyl-5PW, 20 µm	1 L	0014719	38
TSKgel Phenyl-5PW, 20 µm	5 L	0018438	38
TSKgel Phenyl-5PW, 30 µm	25 mL	0043177	38
TSKgel Phenyl-5PW, 30 µm	250 mL	0014720	38
TSKgel Phenyl-5PW, 30 µm	1 L	0014721	38
TSKgel Phenyl-5PW, 30 µm	5 L	0017210	38
TSKgel SP-3PW, 30 µm	25 mL	0021976	14
TSKgel SP-3PW, 30 µm	250 mL	0021977	14
TSKgel SP-3PW, 30 µm	1 L	0021978	14
TSKgel SP-3PW, 30 µm	5 L	0021979	14
TSKgel SP-3PW, 30 µm	50 L	0021980	14
TSKgel SP-5PW, 20 µm	25 mL	0043382	14
TSKgel SP-5PW, 20 µm	250mL	0014714	14
TSKgel SP-5PW, 20 µm	1 L	0014715	14
TSKgel SP-5PW, 20 µm	5 L	0018435	14
TSKgel SP-5PW, 30 µm	25 mL	0043282	14
TSKgel SP-5PW, 30 µm	250 mL	0014716	14
TSKgel SP-5PW, 30 µm	1 L	0014717	14
TSKgel SP-5PW, 30 µm	5 L	0018384	14
TSKgel SP-5PW, 30 µm	50 L	0021807	14
TSKgel SuperQ-5PW, 20 µm	25 mL	0043383	14

Description	Container Size	Part #	Page
TSKgel SuperQ-5PW, 20 µm	250 mL	0018535	14
TSKgel SuperQ-5PW, 20 µm	1 L	0018546	14
TSKgel SuperQ-5PW, 20 µm	5 L	0018547	14
TSKgel SuperQ-5PW, 20 µm	25 L	0021919	14
TSKgel SuperQ-5PW, 20 µm	50 L	0021920	14
TSKgel SuperQ-5PW, 30 µm	25 mL	0043283	14
TSKgel SuperQ-5PW, 30 µm	250 mL	0018536	14
TSKgel SuperQ-5PW, 30 µm	1 L	0018548	14
TSKgel SuperQ-5PW, 30 µm	5 L	0018549	14

Process Development Columns

Description	Container Size	Part #	Page
ToyoScreen AF-Blue HC-650M, 1 mL	1 mL × 6 ea	0021386	83
ToyoScreen AF-Blue HC-650M, 5 mL	5 mL × 6 ea	0021387	83
ToyoScreen AF-Chelate-650M, 1 mL	1 mL × 6 ea	0021384	83
ToyoScreen AF-Chelate-650M, 5 mL	5 mL × 6 ea	0021385	83
ToyoScreen AF-Heparin HC-650M, 1 mL	1 mL × 6 ea	0021390	83
ToyoScreen AF-Heparin HC-650M, 5 mL	5 mL × 6 ea	0021391	83
ToyoScreen AF-Red-650ML, 1 mL	1 mL × 6 ea	0021388	83
ToyoScreen AF-Red-650ML, 5 mL	5 mL × 6 ea	0021389	83
ToyoScreen AF-rProtein A HC-650F, 1 mL	1 mL × 5 ea	0023430	83
ToyoScreen AF-rProtein A HC-650F, 5 mL	5 mL × 1 ea	0023431	83
ToyoScreen AF-rProtein A HC-650F, 5 mL	5 mL × 5 ea	0023432	83
ToyoScreen AF-rProtein A-650F, 1 mL	1 mL × 5 ea	0022809	83
ToyoScreen AF-rProtein A-650F, 5 mL	5 mL × 1 ea	0022810	83
ToyoScreen AF-rProtein A-650F, 5 mL	5 mL × 5 ea	0022811	83
ToyoScreen Butyl-600M, 1 mL	1 mL × 6 ea	0021494	82
ToyoScreen Butyl-600M, 5 mL	5 mL × 6 ea	0021495	82
ToyoScreen Butyl-650M, 1 mL	1 mL × 6 ea	0021376	82
ToyoScreen Butyl-650M, 5 mL	5 mL × 6 ea	0021377	82
ToyoScreen CM-650M, 1 mL	1 mL × 6 ea	0021366	82
ToyoScreen CM-650M, 5 mL	5 mL × 6 ea	0021367	82
ToyoScreen DEAE-650M, 1 mL	1 mL × 6 ea	0021360	82
ToyoScreen DEAE-650M, 5 mL	5 mL × 6 ea	0021361	82
ToyoScreen Ether-650M, 1 mL	1 mL × 6 ea	0021372	82
ToyoScreen Ether-650M, 5 mL	5 mL × 6 ea	0021373	82
ToyoScreen GigaCap CM-650M, 1 mL	1 mL × 6 ea	0021951	82
ToyoScreen GigaCap CM-650M, 5 mL	5 mL × 6 ea	0021952	82
ToyoScreen GigaCap DEAE-650M, 1 mL	1 mL × 6 ea	0022872	82
ToyoScreen GigaCap DEAE-650M, 5 mL	5 mL × 6 ea	0022873	82
ToyoScreen GigaCap Q-650M, 1 mL	1 mL × 6 ea	0021859	82
ToyoScreen GigaCap Q-650M, 5 mL	5 mL × 6 ea	0021860	82
ToyoScreen GigaCap S-650M, 1 mL	1 mL × 6 ea	0021868	82
ToyoScreen GigaCap S-650M, 5 mL	5 mL × 6 ea	0021869	82
ToyoScreen Hexyl-650C, 1 mL	1 mL × 6 ea	0021378	82
ToyoScreen Hexyl-650C, 5 mL	5 mL × 6 ea	0021379	82
ToyoScreen HIC Mix Pack, 1 mL	1 mL × 6 Grades × 1 ea	0021398	83
ToyoScreen HIC Mix Pack, 5 mL	5 mL × 6 Grades × 1 ea	0021399	83
ToyoScreen IEC Anion Mix Pack, 1 mL	1 mL × 3 Grades × 2 ea	0021392	83
ToyoScreen IEC Anion Mix Pack, 5 mL	5 mL × 3 Grades × 2 ea	0021393	83
ToyoScreen IEC Cation Mix Pack, 1 mL	1 mL × 3 Grades × 2 ea	0021394	83
ToyoScreen IEC Cation Mix Pack, 5 mL	5 mL × 3 Grades × 2 ea	0021395	83
ToyoScreen IEC Mix Pack, 1 mL	1 mL × 6 Grades × 1 ea	0021396	83
ToyoScreen IEC Mix Pack, 5 mL	5 mL × 6 Grades × 1 ea	0021397	83
ToyoScreen MegaCap II SP-550EC, 1 mL	1 mL × 6 ea	0021870	82
ToyoScreen MegaCap II SP-550EC, 5 mL	5 mL × 6 ea	0021871	82
ToyoScreen MX-Trp-650M, 1 mL	1 mL × 6 ea	0022824	83
ToyoScreen MX-Trp-650M, 5 mL	5 mL × 6 ea	0022825	83
ToyoScreen Phenyl-600M, 1 mL	1 mL × 6 ea	0021892	83
ToyoScreen Phenyl-600M, 5 mL	5 mL × 6 ea	0021893	83
ToyoScreen Phenyl-650M, 1 mL	1 mL × 6 ea	0021374	83
ToyoScreen Phenyl-650M, 5 mL	5 mL × 6 ea	0021375	83
ToyoScreen PPG-600M, 1 mL	1 mL × 6 ea	0021380	83
ToyoScreen PPG-600M, 5 mL	5 mL × 6 ea	0021381	83
ToyoScreen Q-600C AR, 1 mL	1 mL × 6 ea	0021992	82
ToyoScreen Q-600C AR, 5 mL	5 mL × 6 ea	0021993	82
ToyoScreen QAE-550C, 1 mL	1 mL × 6 ea	0021364	82
ToyoScreen QAE-550C, 5 mL	5 mL × 6 ea	0021365	82
ToyoScreen SP-550C, 1 mL	1 mL × 6 ea	0021370	82
ToyoScreen SP-550C, 5 mL	5 mL × 6 ea	0021371	82
ToyoScreen SP-650M, 1 mL	1 mL × 6 ea	0021368	82
ToyoScreen SP-650M, 5 mL	5 mL × 6 ea	0021369	82
ToyoScreen SuperButyl-550C, 1 mL	1 mL × 6 ea	0021382	83
ToyoScreen SuperButyl-550C, 5 mL	5 mL × 6 ea	0021383	83
ToyoScreen SuperQ-650M, 1 mL	1 mL × 6 ea	0021362	82
ToyoScreen SuperQ-650M, 5 mL	5 mL × 6 ea	0021363	82

Process Development Accessories

Description	Part #	Page
Adapter, 1/4-28 interior to 10-32 exterior, PEEK	0042197	83
Adapter, M6 interior to 10-32 exterior, PEEK	0042196	83
Column Coupler, 10-32, 0.03"ID SS Tubing	0042195	83
ToyoScreen Column Holder	0021400	83
ToyoScreen Holder with fittings	0042194	83

ToyoScreen RoboColumns

Description	Package Description	Part #	Page
Array plate		0045099	84
TOYOPEARL AF-rProtein A-650F RoboColumn	8 × 200 µL	0045061	84
TOYOPEARL AF-rProtein A-650F RoboColumn	8 × 600 µL	0045062	84
TOYOPEARL Butyl-600M RoboColumn	8 × 200 µL	0045033	84
TOYOPEARL Butyl-600M RoboColumn	8 × 600 µL	0045034	84
TOYOPEARL GigaCap CM-650M RoboColumn	8 × 200 µL	0045005	84
TOYOPEARL GigaCap CM-650M RoboColumn	8 × 600 µL	0045006	84
TOYOPEARL GigaCap Q-650M RoboColumn	8 × 200 µL	0045003	84
TOYOPEARL GigaCap Q-650M RoboColumn	8 × 600 µL	0045004	84
TOYOPEARL GigaCap S-650M RoboColumn	8 × 200 µL	0045001	84
TOYOPEARL GigaCap S-650M RoboColumn	8 × 600 µL	0045002	84
TOYOPEARL MX-Trp-650M RoboColumn	8 × 200 µL	0045051	84
TOYOPEARL MX-Trp-650M RoboColumn	8 × 600 µL	0045052	84
TOYOPEARL Phenyl-600M RoboColumn	8 × 200 µL	0045031	84
TOYOPEARL Phenyl-600M RoboColumn	8 × 600 µL	0045032	84
TOYOPEARL Phenyl-650M RoboColumn	8 × 200 µL	0045037	84
TOYOPEARL Phenyl-650M RoboColumn	8 × 600 µL	0045038	84
TOYOPEARL PPG-600M RoboColumn	8 × 200 µL	0045035	84
TOYOPEARL PPG-600M RoboColumn	8 × 600 µL	0045036	84
TOYOPEARL Q-600C AR RoboColumn	8 × 200 µL	0045011	84
TOYOPEARL Q-600C AR RoboColumn	8 × 600 µL	0045012	84

TOYOPEARL LabPak Media

Description	Package Description	Part #	Page
TOYOPEARL AFFIPAK (AF-Amino, Carboxy, Formyl-650M), 65 µm	3 × 10 mL	0043410	85
TOYOPEARL AFFIPAK ACT (AF-Epoxy, Tresyl-650M), 65 µm	2 × 5 g*	0043400	85
TOYOPEARL AIEXPAK (GigaCap Q-650M, SuperQ-650M, Q-600C AR), 65/75/100 µm	3 × 100 mL	0043210	85
TOYOPEARL CIEXPAK (SP-650M, CM-650M, SP-550C), 75/100 µm	3 × 100 mL	0043220	85
TOYOPEARL HICPAK (Ether, Phenyl, Butyl-650M), 65 µm	3 × 25 mL	0019806	85
TOYOPEARL HICPAK HP (Ether, Phenyl, Butyl-650S), 35 µm	3 × 25 mL	0043150	85
TOYOPEARL HICPAK-C (Phenyl, Butyl, Hexyl-650C), 100 µm	3 × 25 mL	0043125	85
TOYOPEARL IEXPAK HP (DEAE-650S, SP-650S, CM-650S, SuperQ-650S), 35 µm	4 × 25 mL	0019817	85
TOYOPEARL SECPAK HMW (HW-55, 65, 75F), 45 µm	3 × 150 mL	0019819	85
TOYOPEARL SECPAK HP (HW-40, 50, 55, 65S), 30 µm	4 × 150 mL	0019820	85
TOYOPEARL SECPAK LMW (HW-40, 50, 55F), 45 µm	3 × 150 mL	0019821	85

TSKgel LabPak Media

Description	Package Description	Part #	Page
TSKgel HICPAK PW (Ether-5PW, Phenyl-5PW), 20 µm	2 × 25 mL	0043150	85
TSKgel HICPAK PW (Ether-5PW, Phenyl-5PW), 30 µm	2 × 25 mL	0019806	85
TSKgel IEXPAK PW (DEAE-5PW, SP-5PW, SuperQ-5PW), 20 µm	3 × 25 mL	0043380	85
TSKgel IEXPAK PW (DEAE-5PW, SP-5PW, SuperQ-5PW), 30 µm	3 × 25 mL	0043280	85

Resin Seeker 96-well plate kits

Description	Package Description	Part #	Page
AIEX 96-well plate kits	Mixed anion exchange plate (20 µL resin beds)	OC41MDAEX-96	86
CIEX 96-well plate kits	Mixed cation exchange plate (20 µL resin beds)	OC41MDCEX-96	86
HIC 96-well plate kits	Mixed hydrophobic interaction plate (20 µL resin beds)	OC41MDHIC-96	86
MMC 96-well plate kits	TOYOPEARL MX-Trp-650M plate (20 µL resin beds)	OC41MDTRP-96	86

TOYOPEARL protein A ELISA kits

Description	Part #	Page
TOYOPEARL AF-rProtein A-650F ELISA	0022815	91
TOYOPEARL AF-rProtein A HC-650F ELISA	0023433	91

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Performance data were determined in Tosoh Corporation.

Printed in Japan

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